IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	Attorney Docket No. Plovin 1-A
Wolfgang HEIL et al.	:	Examiner: M. Bahar
Serial No.: 09/654,227	:	Group: 1617
Filed: August 31, 2000	:	

#22
HKD
3.19.03

For: **PHARMACEUTICAL COMPOSITION FOR USE AS A CONTRACEPTIVE**

DECLARATION UNDER 37 C.F.R. §1.132

SIR:

1. I, Ralph Lipp, being duly warned, declare that:

2. I am a citizen of Germany, residing in Berlin, Germany.

3. I am an inventor of the above-captioned application and am, therefore, familiar with the invention described therein. I am an employee of the assignee, Schering AG, Berlin, Germany. Under German law, I receive royalties from the commercial sale of products covered by this application.

4. Please find attached (as Appendix C) my curriculum vitae showing my expertise in the area of pharmaceuticals.

5. I have read the Office Action mailed May 7, 2002, from the U.S. Patent and Trademark Office, and the references cited therein.

6. I do not consider that one of ordinary skill in the art would have been motivated by

the cited Gast references (WO 98/04267 and 98/04269) or any other prior art of which I am aware to use drospirenone in micronized form for oral administration according to our invention.

7. I respectfully disagree with the statements in the Office Action that: a) it would have been obvious to one of ordinary skill in the art at the time the invention was made to employ drospirenone in micronized form, b) micronization of drospirenone would have been expected to increase its rate of dissolution in vitro, c) one of ordinary skill in the art would have been motivated to employ any known pharmaceutical actives in micronized form merely because variations or optimizations of the dosage regimens are considered within the skill of the artisan, or d) one of ordinary skill would have expected *a priori* that micronization would result in increased bioavailability of drospirenone.

8. Bioavailability of a drug is affected by many factors. Merely providing a drug in a form which exposes more available surface area of the drug cannot reasonably be expected to increase bioavailability or otherwise be advantageous in all cases. Micronization in many cases increases the solubility of a drug, but this is not true in all cases. (See, e.g., Appendix B, References 1 and 2). Moreover, solubility and bioavailability do not necessarily correspond. When drugs are subject to degradation in an environment upon dissolution, for example, in the gastric (acidic) environment for orally administered drugs, increasing their solubility would logically be expected to lessen bioavailability.

9. Some drugs have instability in certain environments which leads to their degradation, e.g., conversion to inactive derivatives, isomers, etc. Such drugs may need to be protected from destabilizing environments so that degradation is prevented or limited until they reach

an environment in which they are stable and can become bioavailable more effectively. For example, the reported low bioavailability of etoposide upon oral administration was thought to be due, at least in part, to chemical instability at pH 1.3, i.e., the typical acidic pH in the human stomach. Etopside has a degradation half-life of about 2.9 hours at pH 1.3. See attached Appendix B, Reference 3.

10. References 1-2, and 4-10 in Appendix B show that micronization of other drugs does not necessarily lead to increased bioavailability over other forms or can be detrimental to bioavailability.

11. It is well known in the art that orally administered drospirenone has to pass through the stomach and into the intestine to be taken up in a bioavailable manner but one of ordinary skill in the art knew that drospirenone was a drug which had instability in acidic media, i.e., it isomerizes to an inactive form under conditions well known to exist in the acidic stomach. See Nickisch et al., Tetrahedron Letters, vol. 27, no. 45, pp. 5463-5466 (1986), translated copy attached. On page 2 of the translation, it is shown that the isomerization results in predominantly (8:2 ratio) the inactive isomerization product in a pH 1 environment such as the stomach. We have further demonstrated that micronized drospirenone has a short degradation/isomerization half-life of about 30 minutes at pH 1, i.e., the isomerization is fast. See attached Appendix A, part 1, showing that when micronized drospirenone is exposed to an acidic environment of pH 1, in vitro, about 50% of the active form of drospirenone is isomerized to its inactive form within 31 minutes. Thus, if providing drospirenone for oral administration in micronized form could have been expected to increase its solubility, as suggested by the Examiner in the Office Action, such increased dissolution would have been expected by one of ordinary skill in the art to expose more of the drospirenone to rapid

isomerization to its inactive form in the stomach. See also Figure 1 of the captioned application for a comparison of the *in vitro* dissolution profiles for micronized (curves V1-V7) versus macrocrystalline (curve V8) drospirenone. Accordingly, one of ordinary skill in the art would not have been motivated to provide orally administrable doses of drospirenone in micronized form, as recited in the claims of the above-captioned application.

12. In summary, based on the above-established facts, one of ordinary skill in the art could not have been motivated to provide and use drospirenone in micronized form as a drug for oral administration. There would have been no reasonable expectation by one of ordinary skill in the art that micronization would increase its bioavailability. The teachings in the art that some drugs can be advantageously administered in micronized form would not be considered by one of ordinary skill in the art to be applicable to all drugs, particularly not to drugs as acid sensitive as drospirenone, especially in view of its known isomerization to an inactive form under acidic conditions. Thus, one of ordinary skill in the art would not have been motivated to modify the teachings of the prior art – including the Gast references – to micronize drospirenone.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: March 7, 2003 Signed: Ralph Lipp

Dr. Ralph Lipp

Dr. Ralph Lipp

APPENDIX A

The half-life of micronized drospirenone during dissolution testing at various pH values is presented. The definition of the half-life relates to the time after which the starting concentration of the active form of micronized drospirenone is reduced to 50% because of isomerization into its non-active isomer.

Results:

pH value	Half-life of micronized drospirenone
1	31 min
2	4.7 h
3.5	≈ 50 h
5	≈ 75 h
7	≈ 75 h

The data clearly indicate that the micronized drospirenone is dramatically degraded at low pH values present in the gastric environment. This would direct the person of skill in the art away from orally administering micronized drospirenone.

APPENDIX B

1. Development of a new tablet formulation of theophylline: In vitro and in vivo studies; Montel et al.; *Drug Development and Industrial Pharmacy*, (vol. 9 (3), pp. 399-420, 1983.

Abstract

" Studies on dissolution rate showed that the release of theophylline from tablet A (theophylline of commercial quality) and tablet B (theophylline of selected particle size) was faster than from tablet C (micronized theophylline)... The in vivo study showed that only tablet B has the same bioavailability as an aqueous solution, whilst bioavailability of tablet A and tablet C was lower than that of tablet B and the aqueous solution."

2. Dissolution properties and in vivo behavior of triamterene in solid dispersions with polyethylene glycol; Arias et al; Abstract of *Pharm-Acta-Helv*., (vol. 71, no.4, pp. 229-235 (1996)).

Abstract

"Relative bioavailability...was greater for all of the solid dispersions than micronized triamterene."

"Dissolution efficiency in 30 min. (DE30) increased from 9.84% for micronized triamterene to 18.5-58.2% for physical mixtures and to 25.26 to 86.17% for solid dispersions."

3. Preformulation study of etoposide: Identification of physicochemical characteristics responsible for the low and erratic oral bioavailability of etoposide; Shah et al.; Abstract of *Pharmaceutical Research*, vol. 6, 408-412, May 1989.

Abstract

"It was concluded that the low equilibrium aqueous solubility, slow intrinsic dissolution rate and chemical instability at pH 1.3 may account for the low oral bioavailability."

4. Phase I and pharmacokinetic study of micronized formulation of carboxyamidotriazole, a calcium signal transduction inhibitor:toxicity, bioavailability and the effect of food; Berlin et al.; Abstract of *Clinical Cancer Research*, 2002, Vol. 8(1); pp. 86-94.

Abstract

"The micronized formulation was absorbed more slowly than the gelcap formulation."

5. Efficacy and safety of reformulated, micronized glyburide tablets in patients with non-insulin dependent diabetes mellitus: a multicenter, double-blind, randomized trial; Carlson et al.; Abstract of *Clinical Therapeutics*, 1993, Vol. 15(5), 788-96.

Abstract

"In a double-blind 12-week study, the subjects were randomly assigned to continue receiving 5-mg tablets of original [non-micronized] glyburide [in doses of 5, 10, 15, or 20 mg daily] or to substitute 3-mg tablets of reformulated, micronized glyburide .... Glyburide tablets had been reformulated [by micronization of the active agent] to

improve their bioavailability... The differences [in serum glucose levels] between groups were not significant."

6. About a Pharmacokinetic Study of Progesterone in Comelts; Duclos et al., Abstract of *Eur. J. Metab. Pharmacokinetic*; 15(2), Suppl., Abstr.226, 1990.

Abstract

"In vitro dissolution rate of progesterone was faster from PEG 600 solid dispersions than from micronized progesterone."

"...solid dispersions gave higher Cmax, earlier Tmax, and increased 8-hour AUC."

7. Bioavailability of griseofulvin from a novel capsule formulation; Fell et al.; Abstract of *The Journal of Pharmacy and Pharmacology*, 1978, 30(8), 479-82.

Abstract

"The in vivo availability of griseofulvin from a novel formulation has been compared with the micronized powder....The results of the in vivo study show the formulation technique has increased the rate and extent of bioavailability of griseofulvin when compared with non-treated (micronized) powder."

8. Lyophilized Preparations of Griseofulvins. 2nd Communication. In vivo release; Froemming et al.; Abstract of *Pharm. Ind.*, 48(7), 1986, 837-40.

Abstract

"Bioavailability of p.o. freeze-dried griseofulvin (GF) was greater than that of ... micronized GF."

9. Comparison of galenic formulations of orlistat (tetrahydrolipstatin). A pharmacological approach. Hartmann et al., Abstract of *Drug Investigation*, 1993, 5(1), 44-50.

Abstract

"...capsule formulations containing orlistat as micronized powder (A) or granules (B) were compared using the following pharmacological end-points..."; "At the 150 mg dose (B) showed a trend toward superior efficacy compared with (A)."

10. Pharmacokinetics and bioavailability of diltiazem. Kohno et al., Abstract of *Arzneimittel Forschung*, 1977, 27(7), 1424-1428.

Abstract

"In the bioavailability study, a comparison of plasma concentrations of diltiazem between the two different crystals and the micronized powder resulted in no difference in their bioavailability."

Appendix C

Curriculum Vitae for Priv. -Doz. Dr. Ralph Lipp

Born: 12 May 1960 in Weiterstadt, Germany
German citizen
Married, two children

Basic studies

1966-79	Elementary and high school
June 1979	High school Graduate

Military service

July 1979-September 1980	Basic military service

Higher education

1980-84	Pharmaceutical Chemistry studies at the University of Mainz
1984	Practical studies in Pharmacy in Weiterstadt
84-85	Practical studies in Röhm Pharma, Weiterstadt
85	Pharmacist graduate
February 1990	Graduation as Doctor in Natural Science at the Free University of Berlin under the guidance of Prof. Dr. Dr. Dr. h. c. W Schunack
2000	International Executive program, INSEAD, Fontainbleau, France
2001	Lecturing exam at the Department of Pharmaceutical Technology at the University of Berlin
2001	Advanced Management Program at Harvard University

Employment Record

85-90	Researcher at the Free University of Berlin in "Instrumental Analysis" and "Drug Formulation"
90-96	Leader of the scientific work group "Dermal and Transdermal Drug Substance Applications" at Schering AG
Since April 1992	Teachers representative at the Department of Pharmaceutical Technology at the Free University of Berlin

96-98 Head of the group "Drug delivery systems- transdermal systems" at Schering

97-2001 Head of Oral Dosage Forms at Schering

Since June 1999 Production manager for clinical test products at Schering AG

Since 2001 Head of Pharmaceutical Development at Schering AG

Professional memberships

INSEAD
Harvard
Member of the German Pharmaceutical chemist's society
and another pharmacist's society

List of publications

Posters and lectures

1. J. Kleine-Tebbe, M. Bolz, R. Lipp, W. Schunack and G. Kunkel, *Presence of histamine-H_3-receptors on human basophils*, Poster, New Engl. Reg. Allergy Proc. 9, Abstract 276 (1988).

2. R. Lipp, W. Schunack, J.-M. Arrang, M. Garbarg and J.-C. Schwartz, *Synthesis and H_3-antagonistic activity of N^{α}-substituted histamine derivatives*, 10th International Symposium on Medicinal Chemistry, Poster, Abstract P-119, Budapest (Hungary), 15.-18.8.1988.

3. J. Kleine-Tebbe, J. Schramm, R. Lipp, W. Schunack and G. Kunkel, *Influence of histamine-H_3-antagonists on human leukocytes*, 18th Meeting of the European Histamine Research Society, Poster, Abstract 119, Breda (Netherlands), 17.-20.5.1989.

4. W. Schunack, S. Elz, F. Keller and R. Lipp, *Chirale Agonisten and Antagonisten des Histamin H_2- and H_3-Rezeptors*. 7. Symposion "Potentielle Arzneistoffe", Lecture, Erfurt (Germany), 24.-26.4.1990.

5. H. Stark, R. Lipp, W. Schunack, J.-M. Arrang, N. Defontaine and J.-C. Schwartz, *Structural variations outgoing from N^{α}-acylated histamine derivatives and their influence on H_3-antagonistic activity*, New Perspectives in Histamine Research, Satellite symposium of the XIth International Congress of Pharmacology of IUPHAR, Poster, Noordwijkerhout (Netherlands), 6.-8.7.1990.

6. J.-M. Arrang, M. Garbarg, J.-C. Schwartz, R. Lipp, H. Stark, W. Schunack and J.-M. Lecomte, *The histamine H_3-receptor: Pharmacology, roles and clinical implications*

studied with agonists, New Perspectives in Histamine Research, Satellite symposium of the XIth International Congress of Pharmacology of IUPHAR, Lecture, Noordwijkerhout (Netherlands), 6.-8.7.1990.

7. R. Lipp, J.-M. Arrang, J. Buschmann, M. Garbarg, P. Luger, W. Schunack and J.-C. Schwartz, *Novel chiral H_3-receptor agonists,* New Perspectives in Histamine Research, Satellite symposium of the XIth International Congress of Pharmacology of IUPHAR, Lecture, Noordwijkerhout (Netherlands), 6.-8.7.1990.

8. J. Kleine-Tebbe, J. Schramm, M. Bolz, H. Gagné, C. Josties, R. Lipp, A. Friese, H. Stark, V. Zingel, A. Buschauer, W. Schunack and G. Kunkel, *Influence of histamine H_1-, H_2-, H_3-(ant)agonists on IgE-mediated histamine release from human basophils*, Poster, International Allergy Congress, München (Germany), 1990.

9. H. Stark, R. Lipp, W. Schunack, J.-M. Arrang, N. Defontaine and J.-C. Schwartz, *Synthese and Aktivität neuer Histamin H_3-Antagonisten*, Scientific congress of the Deutsche Pharmazeutische Gesellschaft (German Pharmaceutical Society), Poster, PA19, Berlin (Germany), 8.-12.9.1990; Arch. Pharm. (Weinheim) 323, 729 (1990).

10. R. Lipp, J.-M. Arrang, J. Buschmann, M. Garbarg, P. Luger, W. Schunack and J.-C. Schwartz, *Synthese, Molekülstruktur and H_3-agonistische Aktiviät seitenkettenverzweigter Histamine*, Scientific congress of the Deutsche Pharmazeutische Gesellschaft (German Pharmaceutical Society), Lecture, DA32, Berlin (Germany), 8.-12.9.1990; Arch. Pharm. (Weinheim) 323, 658 (1990).

11. H. Stark, R. Lipp, W. Schunack, J.-M. Arrang, M. Garbarg and J.-C. Schwartz, *H_3-Activity of alkylated histamine derivatives,* XXth Meeting of the European Histamine Research Society, Poster, P44, Marburg (Germany), 9.-12.5.1991.

12. H. Stark, R. Lipp, W. Schunack, J.-M. Arrang, M. Garbarg, J.-C. Schwartz, *Pharmacochemistry and histamine H_3-activity of alkylhistamines*, United Congress of the French and German Pharmaceutical Societies, Straßburg, France, 19.-22.9.1991.

13. H. Stark, J.-M. Arrang, M. Garbarg, A. Roleau, J.-M. Lecomte, R. Lipp, J.-C. Schwartz and W. Schunack, *Prodrugs of histamine H_3-agonists for improved drug penetration through blood-brain barrier*, XIIth International Symposium on Medicinal Chemistry, Basel (Switzerland), 13.-17.9.1992.

14. H. Stark, J.-M. Arrang, M. Garbarg, A. Roleau, J.-M. Lecomte, R. Lipp, J.-C. Schwartz and W. Schunack, *Prodrug approach for histamine H_3-agonists*, 1st European Congress of Pharmaceutical Sciences, Amsterdam (Netherlands), 7.-9.10.1992.

15. H. Stark, R. Lipp, J.-M. Arrang, M. Garbarg, A. Rouleau, J.-C. Schwartz and W. Schunack, *New histamine H_3-agonistic compoands penetrating into CNS*, XXIInd Annual Meeting of the European Histamine Research Society, Poster, P72, Köln (Germany), 19.-22.5.1993.

16. R. Lipp, *Selection and use of crystallization inhibitors for steroid loaded transdermal delivery systems*, 40th Annual Meeting of the APV, Lecture, Abstract 114, Mainz (Germany), 9.-12. 3. 1994; Eur. J. Pharm. Biopharm. 40 (Suppl.), 85 (1994).

17. R. Lipp, J. Riedl, A. Sachse and T. Schneider, *Cyproteron acetate-containing liposomes for topical application*, 2nd European Congress of Pharmaceutical Sciences, Lecture, FC6, Berlin (Germany), 29.9.-1.10.1994; Eur. J. Pharm. Sci. 2, 102 (1994).

18. R. Lipp and A. Müller-Fahrnow, *X-ray structure determinations of crystals grown in transdermal delivery systems containing estradiol or gestodene*, American Association of Pharmaceutical Scientists Ninth Annual Meeting, Poster, PDD 7154, San Diego (CA, U.S.A.), 6.-10.11.94; Pharm. Res. 11, S-213 (1994).

19. C. Günther, R. Lipp, J. Riedl and U. Täuber, *In vitro studies on the percutaneous absorption of Lisuride*, Prediction of Percutaneous Penetration - Methods Measurements Modeling, Poster, La Grande Motte (France), 2.4.-6.4.1995.

20. C. Günther, R. Lipp, T. Mager, J. Riedl and U. Täuber, *Percutaneous absorption of lisuride in man*, Prediction of Percutaneous Penetration - Methods Measurements Modeling, Oral Poster, La Grande Motte (France), 2.4.-6.4.1995.

21. R. Lipp, H. Laurent, C. Günther, J. Riedl, P. Esperling and U. Täuber, *Rational Design of Prodrugs for Matrix-type Transdermal Delivery Systems: Gestodene Esters*, Symposium on Controlled Release of Bioactive Materials, Seattle 1995, Poster; Proceed. Intern. Symp.Control. Rel. Bioact. Mater., 22, 672 (1995).

22. R. Lipp, *Neue technologische Konzepte für die Entwicklung sexualsteroidhaltiger Transdermalsysteme*, Lecture, Freie Universität Berlin, Berlin (Germany) 1995.

23. R. Lipp, *Transdermal Drug Delivery Systems*, Lecture within the seminar: *Medical Adhesives: Technology and Applications*, Zürich (Switzerland), 2. - 4.12.1996.

24. R. Lipp, *Transdermal Drug Delivery Systems*, Lecture within the seminar: *Medical Adhesives: Technology and Applications*, Basel, (Switzerland) 27.-29.10.1997.

25. R. Lipp and C. Günther, *Use of Dimethylisosorbide to enhance the transdermal fluxes of sex steroids from polyacrylate based matrix TDDS*, American Association of Pharmaceutical Scientists 13th Annual Meeting, Poster, San Diego (CA, U.S.A.), 15.-19.11.1998; Pharm. Sci. 1, (1998).

26. A. P. Funke, C. Günther, R. H. Müller and R. Lipp, *Low-frequency sonophoresis of methyl nicotine at physiological skin temperature*, Poster, PPP-MMM-Conference, 2000.

27. R. Lipp, *Zukunftsweisende Darreichungsformen für Proteine und Peptide*, Lecture, Freie Universität Berlin, Berlin (Germany) 12.02.2001.

28. R. Lipp, *Fortschritte bei steroidhaltigen Drug Delivery Systemen*, Lecture, German Pharmaceutical Society, Berlin (Germany) 25.10.2001.

29. P. Lienau, T. Backensfeld, W. Weitschies and R. Lipp, *The use of phasediagrams in the formulation of self micro-emulsifying systems (SMES) with different types of nonionic surfactants*, Poster, 4th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Florence (Italy) 08.04.-11.04.2002.

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1. J.-M. Arrang, M. Garbarg, W. Schunack, J.-C. Schwartz and R. Lipp, *Composition pharmaceutique contenant des dérives de l'histamine*, Demande de brevet européen (Europ. patent application) 0 214 058 (1.9.1986); US patent 4 767 778 (30.8.1988).

2. J. Altman, M. Wilchek, R. Lipp and W. Schunack, *An improved synthesis of L-homohistidine*, Synth. Comm. 19, 2069 (1989).

3. J.-M. Arrang, M. Garbarg, W. Schunack, J.-C. Schwartz and R. Lipp, *Dérivé de l'histamine, sa préparation et son application en thérapeutiques,* Demande de brevet européen (Europ. patent application) 4 767 778 (25.10.89).

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6. H. Stark, R. Lipp, W. Schunack, J.-M. Arrang, N. Defontaine and J.-C. Schwartz, *Structural variations outgoing from N^{α}-acylated histamine derivatives and their influence on H_3-antagonistic activity*, Agents Actions Suppl. 33, in "New Perspectives in Histamine Research", H. Timmerman and H. van der Goot (Editor), Birkhäuser Verlag, Basel - Boston - Berlin, 1991.

7. J.-M. Arrang, M. Garbarg, J.-C. Schwartz, R. Lipp, H. Stark, W. Schunack and J.-M. Lecomte, *The histamine H_3-receptor: Pharmacology, roles and clinical implications studied with agonists.* Agents Actions Suppl. 33, in "New Perspectives in Histamine Research", H. Timmerman and H. van der Goot (Editor), Birkhäuser Verlag, Basel - Boston - Berlin, 55-67, 1991.

8. R. Lipp, J.-M. Arrang, J. Buschmann, M. Garbarg, P. Luger, W. Schunack and J.-C. Schwartz, *Novel chiral H_3-receptor agonists,* Agents Actions Suppl. 33, in "New Perspectives in Histamine Research", H. Timmerman and H. van der Goot (Editor), Birkhäuser Verlag, Basel - Boston - Berlin, 277-282, 1991.

9. J. Kleine-Tebbe, J. Schramm, M. Bolz, H. Gagné, C. Josties, R. Lipp, A. Friese, H. Stark, V. Zingel, A. Buschauer, W. Schunack and G. Kunkel, *Influence of histamine H_1, H_2-, H_3-(ant)agonists on IgE-mediated histamine release from human basophils*,

in "New Trends in Allergy III", J. Ring and B. Przybilla (Editor), Springer Verlag, Berlin - Heidelberg, 152-157, 1991.

10. M. Garbarg, J.-M. Arrang, W. Schunack, R. Lipp, H. Stark, J.-M. Lecomte and J.-C. Schwartz, *Novel histamine H_3-receptor agonist compoands for therapeutic use, pharmaceutical compositions acting as agonist of said receptor and method of preparation*, WO 91/17146 (14.11.1991); US patent 5342960 (30.8.1994).

11. M. Garbarg, J.-M. Arrang, C. Llorens-Cortes, H. Pollard, A. Roleau, J.-C. Schwartz, M. D. Trung Tuong, R. Lipp, H. Stark, W. Schunack and J.-M. Lecomte, *Autoreceptors and heteroreceptors evidenced by histamine H_3 receptor ligands*, in "Advances in the biosciences", Vol. 82 der Reihe: Presynaptic receptors and neuronal transporters, A. M. Galzin and J. Constantin (Editor), Pergamon Press, Oxford, 67-70, 1992.

12. R. Lipp, H. Stark and W. Schunack, *Pharmacochemistry of histamine H_3-receptors*, in "The histamine receptor", J.-C. Schwartz and H. L. Haas (Editor), Wiley-Liss Inc. New York, 57-72, 1992.

13. J. Riedl, C. Günther and R. Lipp, *Mittel zur transdermalen Applikation enthaltend Ergolin-Derivate*, German patent application DE 4 116 912 (26.11.1992).

14. R. Lipp, J.-M. Arrang, M. Garbarg, P. Luger, J.-C. Schwartz and W. Schunack, *Synthesis, absolute configuration, stereoselectivity, and receptor selectivity of (αR,βS)-α,α-dimethylhistamine, a novel highly potent histamine H_3 receptor agonist*, J. Med. Chem. 35, 4434-4441 (1992).

15. J. Riedl, R. Lipp and M. Hartisch, *Transdermale Therapeutische Systeme mit Penetrationsverstärkern*, German patent application DE 4 210 165 (4.2.1993).

16. R. Lipp, J. Riedl and J. W. Tack, *Transdermale Therapeutische Systeme mit Kristallisationsinhibitoren,* WO 93/08795 (13.5.1993).

17. J.-C. Schwartz, J.-M. Arrang, M. Garbarg, J.-M. Lecomte, C. R. Ganellin, A. Fkyerat, W. Tertiuk, W. Schunack, R. Lipp, H. Stark and K. Purand, *Nouveaux dérivés de l'imidazole, leur preparation et leurs applications thérapeutiques,* French patent application FR 2 686 084 - A1 (16.7.1993).

18. R. Lipp, C. Günther, J. Riedl and U. Täuber, *Transdermal application agent containing 3-Keto-Desogestrel*, International patent application WO 94/04157 (3.3.1994).

19. C. Günther, R. Lipp, U. Täuber and J. Riedl, *Transdermal application agent containing 14a,17α-Ethanoestra-1,3,5(10)-trien-3,17β-diol*, German patent application DE-A 4 240 806 (9.6.1994).

20. H. Stark, R. Lipp, J.-M. Arrang, M. Garbarg, J.-C. Schwartz and W. Schunack, *Acylated and alkylated histamine derivatives as new histamine H_3-receptor antagonists*, Eur. J. Med. Chem. - Chim. Ther. 29, 695-700 (1994).

22. R. Lipp, H. Laurent, C. Günther, J. Riedl, P. Esperling and U. Täuber, *Mittel zur transdermalen Applikation enthaltend Gestodenester*, International patent application WO 95/05827 (2.3.1995).

23. R. Lipp, H. Stark, J.-M. Arrang, M. Garbarg, J.-C. Schwartz and W. Schunack, *Synthesis and histamine H_3-receptor activity of mono- and dialkyl substituted histamine derivatives,* Eur. J. Med. Chem. - Chim. Ther., 30, 219-225 (1995).

24. H. Stark, R. Lipp, J.-M. Arrang, M. Garbarg, X. Ligneau, J.-C. Schwartz and W. Schunack, *New potent histamine H_3-receptor antagonists of the amide type*, Eur. J. Pharm. Sci. 3, 95 (1995).

25. R. Lipp, C. Günther, J. Riedl and U. Täuber, *Desogestrel-containing transdermal application agent*, Europ. patent application EP 95/00481 (09.02.1995).

26. C. Günther, R. Lipp, J. Riedl and U. Täuber, *In vitro studies on the percutaneous absorption of Lisuride*, Brain, K. R.; James, V. J. and Walters, K. A. (Editor) Prediction of Percutaneous Penetration – Methods Measurements Modeling, STS Publishing Ltd., Cardiff, UK, 89-92 (1995).

27. C. Günther, R. Lipp, T. Mager, J. Riedl and U. Täuber, *Percutaneous absorption of lisuride in man*, Brain, K. R.; James, V. J. and Walters, K. A. (Editor) Prediction of Percutaneous Penetration – Methods Measurements Modeling, STS Publishing Ltd., Cardiff, UK, 85-88 (1995).

28. M. Krause, A. Roleau, H. Stark, P. Luger, R. Lipp, M. Garbarg, J.-C. Schwartz and W. Schunack, *Synthesis, X-ray crystallography and pharmacology of novel azomethine prodrugs of (αR)-α-methylhistamine: highly potent and selective histamine H_3 receptor agonists*, J. Med. Chem. 38, 4070 (1995).

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ACID-CATALYZED REARRANGEMENTS OF 15β,16β-METHYLENE-17α-PREGNENE-21,17-CARBOLACTONE DERIVATIVES

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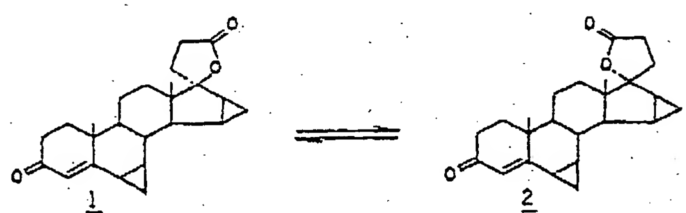
Summary: The different acid-catalyzed rearrangements of 15,16-substituted 17α-pregnene-21,17-carbolactone derivatives are described.

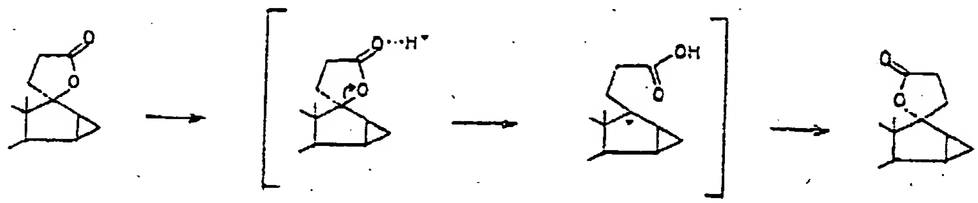
The search for new steroidal aldosterone antagonists with reduced endocrinological side effects has been the subject of intensive efforts in therapy since the introduction of the spironolactone (7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone). The compiled structure-action relationships show that virtually all changes of the 17-spiro-five-ring-lactone result in a reduction of the antimineral-corticoidal action.[1] The tests performed in our laboratories provided the result that the aldosterone-antagonistic action of known compounds can be significantly increased by the anellation of a 15β,16β-cyclopropane ring.[2]

In our synthetic works in this family of substances, we discovered a special reaction behavior of this 15β,16β-methylenespirolactone, on which we would like to report here. In the case of the final purification of several test substances from the series

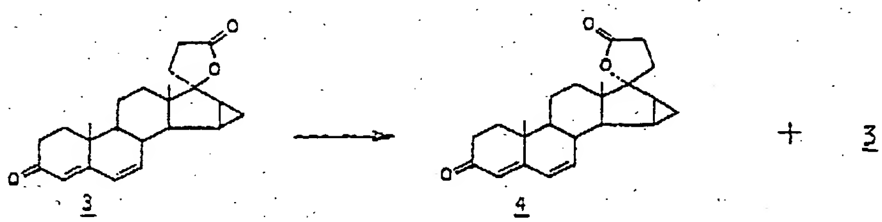
of 15β,16β-methylenespirolactones, by-products were isolated that behave almost identically in terms of chromatography, and their UV and IR spectra are very difficult to distinguish from those of the main products. The nuclear resonance spectra of these by-products show a significant upfield shift of the 18-methyl group by about 0.2 ppm. This shift allows the conclusion that the 18-methyl group and the 17-lactone function are no longer both in β-position. All available spectroscopic data can be best explained with the presence of an isomeric spirolactone. An accurate test, under which conditions this isomerization occurs, provided the result that this is an acid-catalyzed reaction. Thus, 1 could be converted into an 8:2 mixture of 2 and 1 by treatment with 0.1N hydrochloric acid at room temperature within 3 hours.[3]



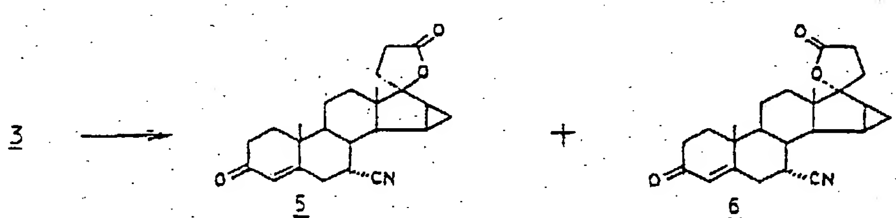
The same mixture is obtained if 2 is treated under identical conditions. The product-ratio 8:2 of compounds 2 and 1 thus represents the thermodynamic equilibrium of the acid-catalyzed isomerization. This rearrangement can be explained mechanistically by the primary protonation of the lactone oxygen, which then results in the formation of a homoallyl cation[4], which in addition is stabilized by the carboxyl group and can be attacked innermolecularly by the carboxylic acid from the top side or the bottom side with the formation of lactone rings. The α-attack is promoted because of the β-position of the 15,16-methylene ring and the 18-methyl group.



This rearrangement can be applied in general to 15β,16β-methylenespirolactones. Compound 4 thus can be produced from 3 in dioxane/2N H_2SO_4 9:1 at 60°C.[5]

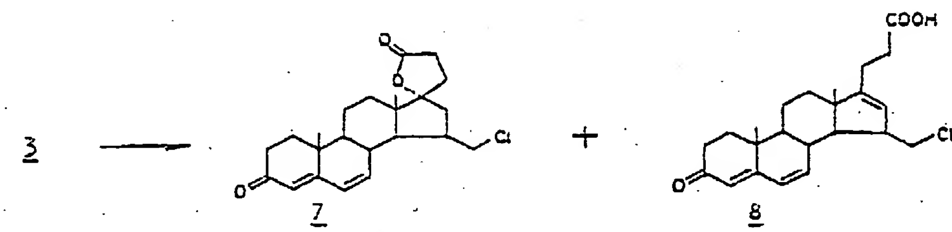


This isomerization can also be catalyzed by Lewis acids. The reaction of 3 with diethylaluminum cyanide in dichloromethane or benzene thus yields a mixture of 7-cyanides 5 and 6, while only 5 can be obtained when the more basic solvent THF is used.



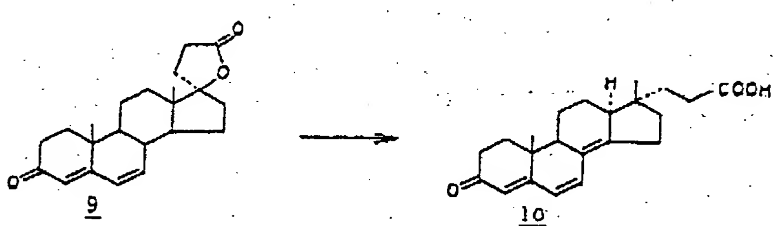
If the isomerization is tested under conditions (acetic acid/concentrated HCl 1:1) that yield Δ^{13}-17,17-dialkyl compounds starting from 17α-alkyltestosterone derivatives with migration of the 18-methyl group, a mixture of isolactone 7 and an acid 8 is

obtained whose structural determination was possible only after esterification with diazomethane.[7]



3 → 7 + 8

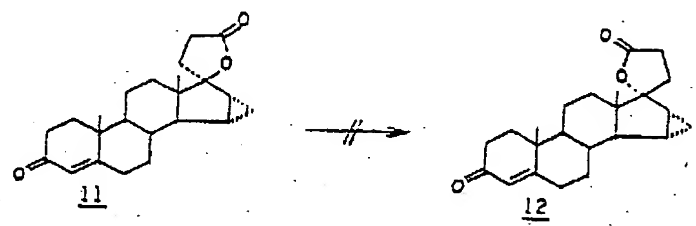
The formation of 7 and 8 can be explained with the nucleophilic chloride attack on the primary formed homoallyl cation. To test the influence of the 15β,16β-cyclopropane ring on the rearrangement, spirolactone 9 that is unsubstituted in 15,16-position was subjected to the acid-catalyzed rearrangement conditions. If compound 9 is reacted under standard conditions (dioxane/2N H_2SO_4 9:1, 60°C), only starting material can be isolated after 72 hours. If the reaction is performed in acetic acid/concentrated HCl 1:1, acid 10 is isolated as a single compound (Flash point: 220-222°C/Lit.[8] 214-216°C).



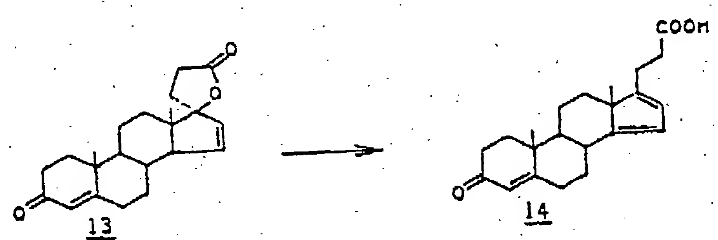
9 → 10

To clarify the question of what influence the substituents in 15,16-position have on the reaction behavior of the spirolactone ring, the 15α,16α-methylene derivative 11 and the Δ^{15}-analog 13 were exposed to the rearrangement conditions. The 15α,16α-

methylenespirolactone 11 itself at 100°C proved to be stable under the standard conditions (dioxane/2N H_2SO_4 9:1). After 28 hours of boiling, only the starting material could be isolated.



In the reaction of the Δ^{15}-compound 13 under standard conditions, however, dienecarboxylic acid 14 was formed.[9]



The formation of a 17-isolactone compound also could not be observed after variation of the reaction conditions. The formation of 14 can be explained by the cleavage of a proton from the intermediately formed allyl cation.

In summary, the varied reaction behavior of the various 15,16-substituted compounds can be described as follows: The higher reactivity of the Δ^{15}- and the 15β,16β-methylene compounds compared to acids can be explained by the slight build-up of allyl or homoallyl cations. Based on the reaction conditions, the carbenium ions that are formed can react off to form different products.

Steric bases may be responsible for the varying reaction behavior of the 15α,16α- and 15β,16β-methylene derivatives. In the case of the 15β,16β-methylene compounds, a substituent cluster is present on the top side of the D ring. This inhibition can be reduced by the formation of a carbenium ion in the 17-position, by which a stabilization of the homoallyl cation is produced. In the case of the 15α,16α-methylene derivatives, this additional stabilization does not take place, i.e., the formation of the carbenium ion is promoted to a lesser extent in terms of energy. The 15α,16α-methylene derivatives are therefore more stable compared to acids.

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5. Flash point: 225-226°C; H-NMR ($CDCl_3$): 0.9 (s, 3, 18-CH_3), 1.13 (s, 3, 19-CH_3) ppm; uv: λ_{max} (ε) 284 (27050); IR (KBr): 1765, 1660, 1620, 1585 cm^{-1}.
6. A. Segaloff and R. B. Gabbard, Steroids 4, 433 (1964).
7. 7: Flash point: 278-280°C;

   H-NMR ($CDCl_3$): 0.98 (s, 3, 18-CH_3), 1.16 (s, 3, 19-CH_3),

3.49 (dd, 12 + 11 Hz, 1) and 3.70 (dd, 12 + 4 Hz, 1) –CH_2Cl,

5.72 (s, 1, H-4), 6.20 (s, 2, H-6 and H-7) ppm;

IR (KBr): 1770, 1665, 1620, 1590 cm^{-1}.

8: (as methyl ester) H-NMR ($CDCl_3$): 1.07 (s, 3, 18-CH_3), 1.18 (s, 3, 19-CH_3),

3.52 (dd, 12 + 11 Hz, 1) and 3.73 (dd, 12 + 4 Hz, 1) –CH_2Cl,

3.7 (s, 3, -$COOCH_3$), 5.58 (s, 1, H-16),

5.69 (s, 1, H-4), 6.2 (m, 2, H-6 and H-7) ppm;

IR (KBr): 1740, 1665, 1620, 1585 cm^{-1}.

8. W. Sadhe, S. Riegelman and L. F. Johnson, Steroids 17, 595 (1971).

9. H-NMR (D_5-pyridine): 1.04 (s, 3, 18-CH_3), 1.10 (s, 3, 19-CH_3), 5.82 (s, 1, H-16),

5.86 (s, 1, H-4), 6.16 (s, 1, H-15) ppm;

UV: λ_{max} (ε) 242 (16500);

IR (KBr): 2500-3000, 1735, 1660, 1620, 1605, 1570 cm^{-1}.

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SÄURE-KATALYSIERTE UMLAGERUNGEN VON 15β,16β-METHYLEN-17α-PREGNEN-21,17-CARBOLACTON-DERIVATEN

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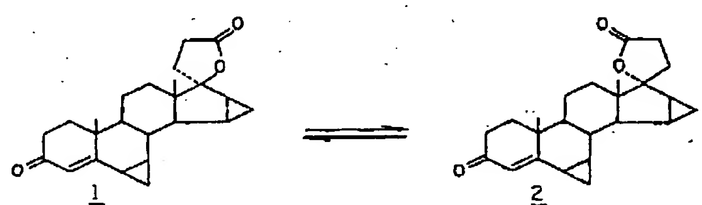
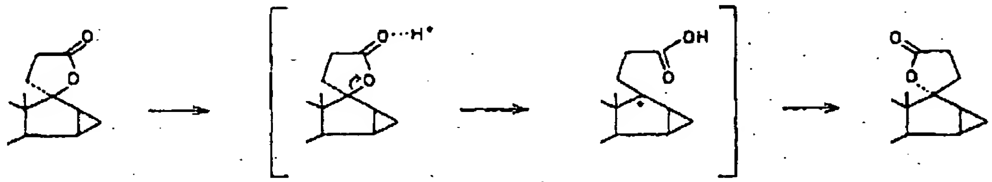
Summary: The different acid catalyzed rearrangements of 15,16-substituted 17α-pregnene-21,17-carbolactone derivatives are described.

Die Suche nach neuen steroidalen Aldosteronantagonisten mit reduzierten endokrinologischen Nebenwirkungen ist seit der Einführung des Spironolactons (7α-Acetylthio-3-oxo-17α-pregn-4-en-21,17-carbolacton) in die Therapie Gegenstand intensiver Bemühungen. Die erarbeiteten Struktur-Wirkungsbeziehungen zeigen, daß praktisch alle Veränderungen des 17-Spirofünfringlactons zu einer Reduzierung der antimineralcorticoiden Wirkung führen.[1] Die in unseren Laboratorien durchgeführten Untersuchungen ergaben, daß die aldosteronantagonistische Wirkung von bekannten Verbindungen durch die Anellierung eines 15β,16β-Cyclopropanringes deutlich gesteigert werden kann.[2]

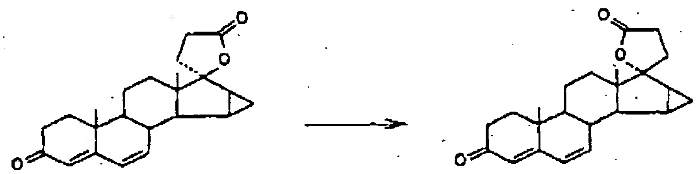
Bei unseren synthetischen Arbeiten in dieser Stoffklasse stießen wir auf ein spezielles Reaktionsverhalten dieser 15β,16β-Methylenspirolactone, über das wir hier berichten wollen. Bei der Endreinigung einiger Testsubstanzen aus der Reihe der 15β,16β-Methylenspirolactone wurden Nebenprodukte isoliert, die sich chromatographisch fast identisch verhielten und deren UV- und IR-Spektren sich kaum von denen der Hauptprodukte unterschieden. Die Kernresonanzspektren dieser Nebenprodukte zeigen einen deutlichen upfield shift der 18-Methylgruppe um ca. 0.2 ppm. Diese Verschiebung läßt den Schluß zu, daß die 18-Methylgruppe und die 17-Lactonfunktion nicht mehr beide β-ständig sind. Alle verfügbaren spektroskopischen Daten lassen sich am besten mit dem Vorliegen eines isomeren Spirolactons erklären. Eine genaue Untersuchung, unter welchen Bedingungen diese Isomerisierung auftritt, ergab, daß es sich um eine säurekatalysierte Reaktion handelt. So konnte 1 durch Behandeln mit 0.1 N Salzsäure bei Raumtemperatur innerhalb von 3 Stunden in ein 8:2 Gemisch von 2 und 1 umgewandelt werden.[3]


1 2

Das gleiche Gemisch erhält man, wenn 2 unter identischen Bedingungen behandelt wird. Das Produkt-Verhältnis 8:2 der Verbindungen 2 und 1 stellt somit das thermodynamische Gleichgewicht der säurekatalysierten Isomerisierung dar. Mechanistisch läßt sich diese Umlagerung über die primäre Protonierung des Lactonsauerstoffs erklären, die dann zur Ausbildung eines Homoallylkations führt[4], das zusätzlich durch die Carboxylgruppe stabilisiert wird und innermolekular von der Carbonsäure von der Ober- oder Unterseite unter Lactonringbildung angegriffen werden kann. Der α-Angriff ist wegen der β-Ständigkeit des 15,16-Methylenringes und der 18-Methylgruppe begünstigt.

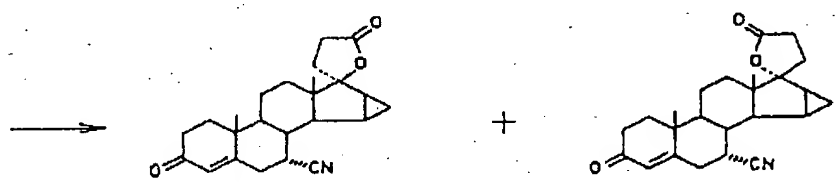


Diese Umlagerung ist allgemein anwendbar auf 15β,16β-Methylenspirolactone. So läßt sich aus 3 die Verbindung 4 in Dioxan/2 N H_2SO_4 9:1 bei 60°C darstellen.[5]



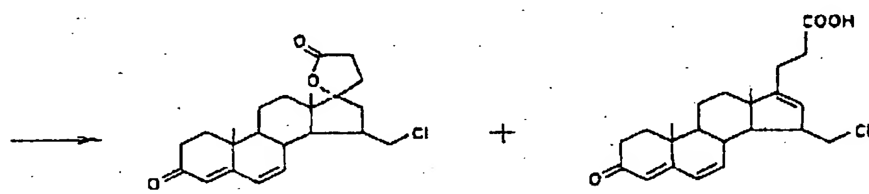
3 4 + 3

Diese Isomerisierung kann auch durch Lewissäuren katalysiert werden. So liefert die Umsetzung von 3 mit Diethylaluminiumcyanid in Dichlormethan oder Benzol ein Gemisch der 7-Cyanide 5 und 6, während bei der Verwendung des basischeren Lösungsmittels THF nur 5 erhalten werden kann.



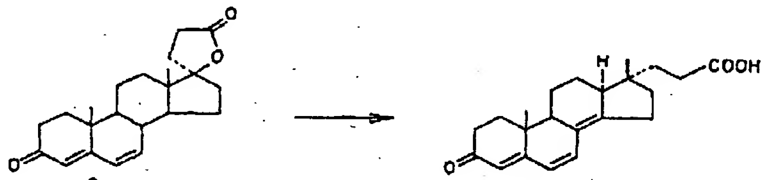
3 5 6

Untersucht man die Isomerisierung unter Bedingungen (Essigsäure/konz. HCl 1:1), die ausgehend von 17α-Alkyltestosteronderivaten unter Wanderung der 18-Methylgruppe Δ^{13}-17,17-Dialkylverbindungen ergeben,[6] so erhält man ein Gemisch des Isolactons 7 und einer Säure 8, deren Strukturaufklärung erst nach der Veresterung mit Diazomethan gelang.[7]



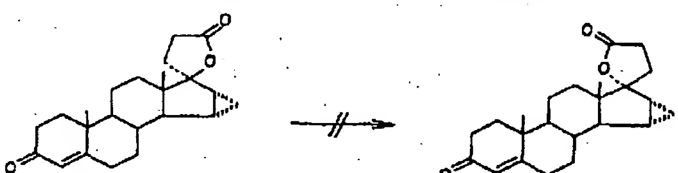
3 7 8

Die Bildung von 7 und 8 läßt sich über den nucleophilen Chloridangriff auf das primär gebildete Homoallylkation erklären. Um den Einfluß des 15ß,16ß-Cyclopropanringes auf die Umlagerung zu untersuchen, wurde das in 15,16-Position unsubstituierte Spirolacton 9 den säurekatalysierten Umlagerungsbedingungen unterworfen. Setzt man Verbindung 9 unter Standardbedingungen (Dioxan/2 N H_2SO_4 9:1, 60°C) um, so kann nach 72 Stunden nur Ausgangsmaterial isoliert werden. Führt man die Reaktion in Essigsäure/konz. HCl 1:1 durch, wird als einzige Verbindung die Säure 10 isoliert (Fp: 220-222°C/Lit.[8] 214-216°C).



9 10

Um die Frage zu klären, welchen Einfluß die Substituenten in 15,16-Position auf das Reaktionsverhalten des Spirolactonringes haben, wurde das 15α,16α-Methylenderivat 11 und das Δ^{15}-Analogon 13 den Umlagerungsbedingungen ausgesetzt. Das 15α,16α-Methylenspirolacton 11 erwies sich unter den Standardbedingungen (Dioxan/2 N H_2SO_4 9:1) selbst bei 100°C als stabil. Nach 28-stündigem Kochen konnte nur Ausgangsmaterial isoliert werden.



11 12

Bei der Umsetzung der Δ^{15}-Verbindung 13 unter Standardbedingungen bildete sich dagegen die Diencarbonsäure 14.[9]

13 14

Die Bildung einer 17-Isolactonverbindung konnte auch nach Variation der Reaktionsbedingungen nicht beobachtet werden. Die Bildung von 14 läßt sich durch die Abspaltung eines Protons aus dem intermediär gebildeten Allylkation erklären.

Zusammenfassend kann man das unterschiedliche Reaktionsverhalten der verschiedenen 15,16-substituierten Verbindungen wie folgt beschreiben: Die höhere Reaktivität der Δ^{15}- und der 15ß,16ß-Methylenverbindungen gegenüber Säuren läßt sich durch die leichte Ausbildung der Allyl- bzw. Homoallylkationen erklären. In Abhängigkeit von den Reaktionsbedingungen können die gebildeten Carbeniumionen zu unterschiedlichen Produkten abreagieren.

Für das unterschiedliche Reaktionsverhalten der 15α,16α- und 15β,16β-Methylenderivate können sterische Gründe verantwortlich sein. Im Falle der 15β,16β-Methylenverbindungen liegt eine Substituentenhäufung auf der Oberseite des D-Ringes vor. Diese Hinderung kann durch die Ausbildung eines Carbeniumions in der 17-Position vermindert werden, wodurch sich eine Stabilisierung des Homoallylkations ergibt. Im Falle der 15α,16α-Methylenderivate entfällt diese zusätzliche Stabilisierung, d.h. die Ausbildung des Carbeniumions ist energetisch weniger bevorzugt. Deshalb sind die 15α,16α-Methylenderivate stabiler gegenüber Säuren.

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DEVELOPMENT OF A NEW TABLET FORMULATION OF THEOPHYLLINE : IN VITRO AND IN VIVO STUDIES

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ABSTRACT

The preparation of a new scored 250 mg theophylline tablet is described, for which effects of particle size of the active principle, aspects of granulation and changes in tabletting settings were investigated.

In vitro studies showed the dissolution rate from tablets prepared from theophylline of commercial quality (50 µm) or of selected particle size (30 µm) to be faster than that from tablets prepared from micronized theophylline (10 µm). In vivo studies in dog showed that only the tablet from theophylline of selected particle size has the same bioavailability as an aqueous solution.

The scale up study showed that the characteristics of the tablets, including dissolution rate, are independent of the formulation factors.

AIMS OF THE STUDY

This work was undertaken to prepare a new theophylline tablet having a rapid rate of dissolution and, if possible, the same bioavailability as an aqueous solution, or one at least equivalent to those of the more efficient formulations available within the EEC. The additional requirements for this formulation were that the tablets should be easy and reproducible to manufacture and that the new formulation should allow the dose of theophylline to be adjusted to the individual needs of the patients by means of scored tablets, possibly even by multiple scoring. The size of the tablet

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was also taken into account to encourage patient compliance during treatment.

MATERIALS AND METHODS

Raw Materials and their Characterization

Anhydrous theophylline[1], which complies with the European Pharmacopoeia monograph; four samples having the following characteristics were investigated :

- a sample of commercial quality, mean particle size 50 µm (batches R966 and R1027),
- a sample having a carefully selected particle size (CSPS) with a mean particle size of 30 µm, obtained from commercial quality theophylline by a controlled milling process (batch R1028),
- a micronized sample, mean particle size 10 µm (batch 51647),
- a spray dried sample, mean particle size 40 µm (batch L8008).

All the other materials conformed to the USP XX and European Pharmacopoeia monographs.

Particle size determination was carried out using an Alpine[2] air jet sifter as described in the French norm AFNOR[3] NFX 11-640. Dissolution profiles for theophylline were measured in 750 ml of 0.1N HCl at 37 $\pm$ 0.5°C using the apparatus 3 described in the USP XX. It was not possible to use the same apparatus for both the raw materials and for the theophylline tablets (apparatus 2), because of "powder-caking". This was in spite of efforts to apply a method published recently[1].

X-Ray diffraction patterns from a powdered specimen under vacuum was obtained with a Guinier de Wolff camera using the Cu Kα radiation at 15.418 nm. The measurement of intensities on the films was by means of a microphotometer.

[1]Finorga, F-38670, Chasse sur Rhône.
[2]Alpine, D-8900, Augsburg.
[3]AFNOR, F-92080, Paris La Défense.

Tablet Preparation

Mixing was by means of a Z arm type mixer[4] having a capacity of 1, 5 or 30 liters, or a 'hurling and whirling' type mixer[5], capacity 50 and 130 liters, drying by fluid bed dryers, first in an Aeromatic[6] ST2, then in an ST15 and granulation with an oscillating granulator (Erweka FGS)[7] fitted with a screen having a 1 mm mesh. Tabletting was carried out with a reciprocating single punch tabletting machine (Frogerais AM)[8] fitted with 11 mm diameter flat punches which were equipped with strain gauges on the upper and lower punches and a displacement transducer on the upper punch. Tablets were also prepared on a rotating 15 stations machine (Frogerais MR15)[8] with 11 mm diameter flat punches equipped with strain gauges on the compression roll.

Tablet Weight was measured for 50 tablets with an accuracy of $\pm$ 0.1 mg on an electronic weighing unit connected to a computer (Mettler HL32[9] + Hewlett Packard 97S[10]) which calculated the variation in weight.

Tablet Hardness was measured on Schleuniger[11] apparatus.

Tablet Friability was measured on 10 tablets by a Roche friabilator during a 15 min period at 30 r.p.m.

Tablet Disintegration was studied as described in the USP XX and the European Pharmacopoeia.

Dissolution Testing was carried out using the USP XX apparatus 2. The medium used was 500 ml of 0.1N HCl at 37 $\pm$ 0.5°C. Paddle

[4]Guittard-Perkins, F-77500, Chelles.
[5]Gebrüder Lödige, D-4790, Paderborn.
[6]Aeromatic AG, CH-4132, Muttenz.
[7]Erweka, D-6056, Heusenstamm.
[8]Ets. Frogerais, F-94596, Rungis.
[9]Mettler AG, CH-8606, Greifensee; Zürich.
[10]Hewlett Packard, OR 97330, Corvallis, USA.
[11]Dr. D. Schleuniger, CH-8033, Zürich.

stirring speed was 56 r.p.m. A 3 ml sample was filtered through a 0.45 µm Millipore membrane filter[12] and diluted. The amount of drug in solution was estimated using an ultraviolet spectrophotometer at 276 nm. Samples withdrawn were not replaced by an equal volume of dissolution medium.

Bioavailability Studies

"In vivo" tests were carried out in 3 beagle dogs weighing 11.8-12.4 kg. Dogs were fasted (water ad libitum) for 12 hr before and 8 hr after drug intake. They received single 250 mg oral doses of theophylline : an aqueous solution (30 ml, batch 1), three tablet formulations prepared with the raw materials tested and two Theolair® tablets[13] (2 x 125 mg, batch 79G03). The administration was according to a cross over design. Tablets were given with 30 ml of water.

Venous blood (6 ml samples) was collected before each administration, then at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 24, 28, 32 and 48 hr after. Blood was immediately centrifuged, plasma was separated and stored at -20°C until analyzed. A period of two weeks was allowed between each administration.

Analytical Method

Theophylline was measured in plasma by High Performance Liquid Chromatography (HPLC). 1 ml of plasma was spiked with 200 µl of an aqueous solution of the internal standard, diazepam (50 mg/l); 1 ml of 1N HCl was added and the solution was extracted with 10 ml of chloroform/isopropanol (95/5, v/v). Tubes were shaken for 30 min then centrifuged at 1000 g. The organic phase was filtered on hydrophobic paper Whatman 1PS[14], then evaporated to dryness at 30°C under vacuum.

[12] Millipore, Mass. 01730 Bedford, USA.

[13] Theolair ®, Riker Laboratories, Brussels, Belgium.

[14] Whatman, F-45210, Ferrières.

The residue was taken up into 150 µl of isopropanol. A Varian Aerograph[15] 8500 HPLC equipped with a Varichrom UV detector set at 270 nm, was used. The stainless steel column (15 cm x 0.46 cm i.d.) was packed with Sil 60 D 5 CN[16]. The mobile phase used was hexane/isopropanol/methanol (90/10/0.5, v/v) at a flow rate of 1 ml/min at 20°C.

Under these conditions, the HPLC retention times were 7 and 8.5 min for diazepam and theophylline respectively.

Quantification of theophylline was obtained by the height ratio method (height theophylline/height diazepam) and the calibration curve gave a linear response for plasma concentrations of theophylline ranging from 0.2 to 40 mg/l. The recovery of theophylline from plasma was 85 ± 5 % and the lower limit of sensitivity was 0.1 mg/l for theophylline and the internal standard. The main theophylline metabolites (3-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid), caffeine and theobromine did not interfere with the assay.

Pharmacokinetic Analysis

Pharmacokinetic analysis of the plasma curves was carried out using the G-PHARM interactive program[2] according to a one compartment open model. The parameters were calculated as follows :

$t_{1/2}$ abs = half-life of the absorption phase (hr)

t_{max} = time of the peak plasma concentration (hr)

C_{max} = peak plasma concentration (mg/l)

$t_{1/2}\ \beta$ = half-life of the elimination phase (hr)

$AUC_\infty = AUC_{48} + \frac{C_{48}}{\beta}$ = area under the plasma concentration/time curve extrapolated to infinity, determined by the trapezoidal rule. AUC_{48} and C_{48} represent the area and the plasma concentration values at 48 hr.

[15] Varian, CA 94303, Palo Alto, USA.

[16] Chrompack, F-91440, Orsay les Ulis.

$$F = \frac{AUC_\infty \text{ tablet}}{AUC_\infty \text{ aqueous solution}} = \text{availability of the tablet relative to the aqueous solution.}$$

Statistical Analysis

A two-way analysis of variance (formulation, dog) was used to compare the values of $t_{1/2}abs$, t_{max}, C_{max} and AUC_∞ obtained after the 5 doses. Since the variance observed for the C_{max} values was not homogeneous (Bartlett's test : $p < 0.05$), this parameter was analyzed by a non parametric test described by Friedman[3]. The AUC_∞ values were compared using symmetrical confidence intervals as for bioequivalence trials[4].

RESULTS AND DISCUSSION

Raw Materials

X-Ray diffraction patterns showed that the crystalline characteristics of the commercially available theophylline products remained the same before and after milling or micronisation. The results of the diffraction study agreed with those reported in the ASTM records 27 - 1977[5].

The physical characteristics of the raw materials were investigated further. The four batches of theophylline were compared by electron microscopy (Fig. 1 a & b), by analysis of particle size and by dissolution kinetics (Fig. 2).

The mean particle size of commercially available theophylline (50 µm) was reduced to about 30 µm by a carefully controlled milling process, but the dissolution kinetics were similar for the two samples.

After micronisation, the mean particle size of theophylline was decreased to 10 µm, however, analysis by electron microscopy (Fig. 1 b) showed agglomerates of the particles (200-300 µm) which could explain the slower rate of dissolution of this theophylline product.

Despite the reduction in particle size obtained after spray drying (40 µm), the dissolution rate of theophylline was slower

than from the other three products considered. This was probably due to a poor wettability of this powder. For this reason and because of problems associated with the industrial preparation, this product was not further investigated.

Formulation

The preparation of 250 mg tablets was commenced by first using commercially available theophylline. After some compressibility tests, the preparation of tablets by a wet granulation process was preferred to that of direct tabletting. In order to improve the compressibility of theophylline and to prepare a tablet having an acceptable disintegration/dissolution performance, several diluents currently in use were tested with different binders at various concentrations : the best results were achieved with calcium phosphate dihydrate as diluent and water as wetting agent. The formulation study was then completed by selection of the disintegrant and lubricant, and the determination of their optimal concentrations. Granulation, drying, lubrication and tabletting were satisfactory as shown by the pharmaceutical characteristics (table 1) and dissolution kinetics (Fig. 3) of the tablet. With the same raw materials and under the same operating conditions, the variability of the results at this early stage of development was low either for the same batch (tests 1 & 2), or for a different batch (test 3) of theophylline.

Compression cycles showed a good compressibility and satisfactory lubrication of the tablet : an example of a typical record is given in figure 4. The shape of the tablets prepared was discoid with a diameter of 11 mm and a thickness of 3.2-3.5 mm. The tablet was scored on one face and can therefore be easily administered or divided into two parts[6, 7].

Under the experimental conditions described above, the dissolution kinetics of the tablet prepared were similar to those of two batches of Theolair® tablets (batch 79 G08 and batch 79 F26) (Fig. 3). A compression test has shown that it is possible to prepare a capsule shaped tablet (17 x 6.4 mm).

FIGURE 1. a.

Electron photomicrographs of theophylline.
1. : commercial quality - 2 : selected particle size

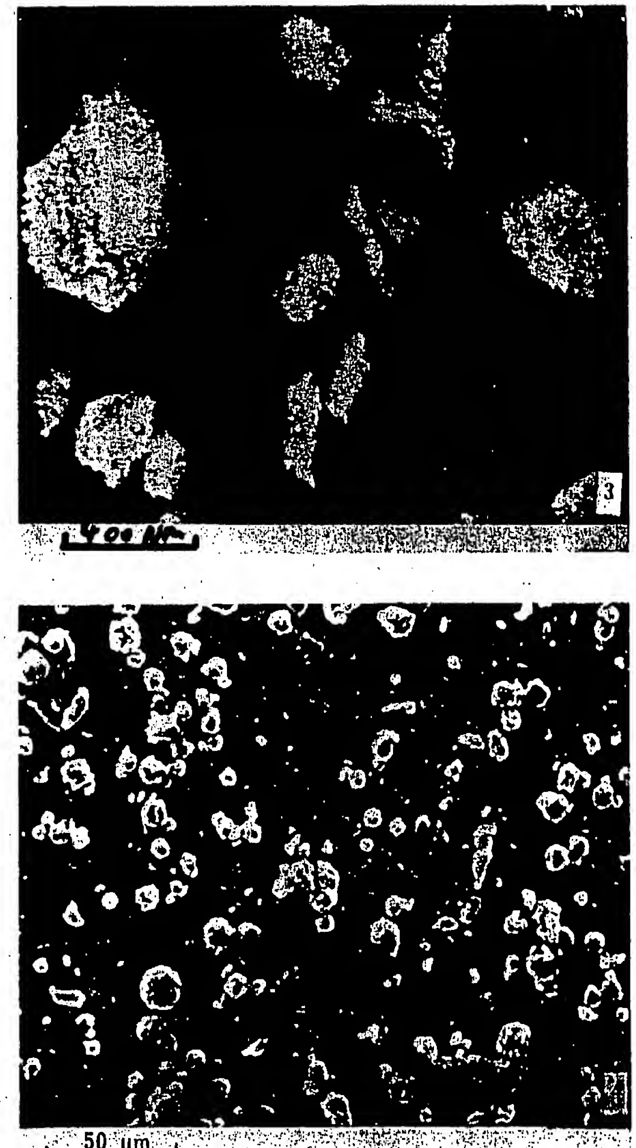

FIGURE 1.b.

Electron photomicrographs of theophylline
3 : micronized - 4 : spray-dried

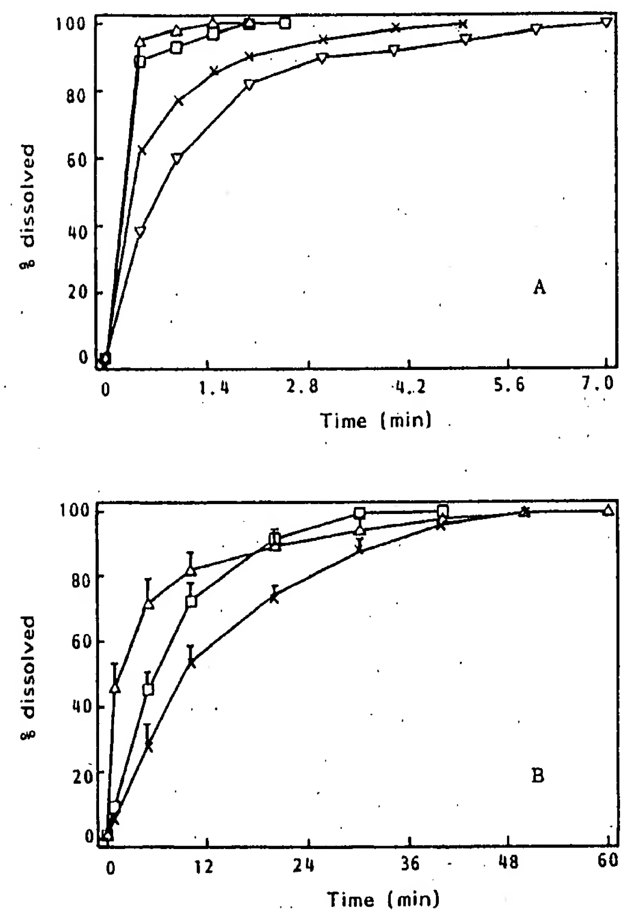

FIGURE 2

Dissolution profiles of theophylline. Commercial quality (Δ), selected particle size (□), micronized (x), spray-dried (∇). A : theophylline raw material; B = theophylline tablets.

TABLE 1

Characteristics of tablets A obtained from 2 batches of theophylline tests 1 & 2 relate to the batch R966 and test 3 to the batch R1027.

	test 1	test 2	test 3
Compression force kN	9.0	8.8	6.6
Hardness daN ± S.D.	14.4 ± 0.5	15.5 ± 0.8	12.4 ± 0.9
Friability %	0.6	0.4	0.5
Mean weight ± S.D.	400.9 ± 2.6	398.9 ± 3.6	405.2 ± 4.4
Weight CV %	0.7	0.8	1.1
Disintegration time (sec)	32	33	32

Influence of Theophylline Batch in the Formulation

At this point of the development, the tablets containing the milled (tablet B) and the micronized theophylline (tablet C) were formulated. The pharmaceutical properties of the two tablets were compared with those obtained with tablets prepared from commercial quality theophylline (tablet A) (table 2).

The results showed that the differences between the three formulations were negligible. As observed for the raw materials, the dissolution rate of tablets A and B was higher than that of tablet C (Fig. 2). On the basis of the dissolution kinetic experiments, the formulations A and B provided a faster release of theophylline.

Bioavailability Study

Before starting the study to scale up the formulation, the "in vivo" characteristics of the three different tablets were investigated. The bioavailability of tablets A, B and C relative to an aqueous solution (250 mg theophylline) and to Theolair® tablets (2 x 125 mg theophylline) was determined in three dogs.

Plasma concentrations of theophylline were best fitted to a one compartment open model. The pharmacokinetic parameters ob-

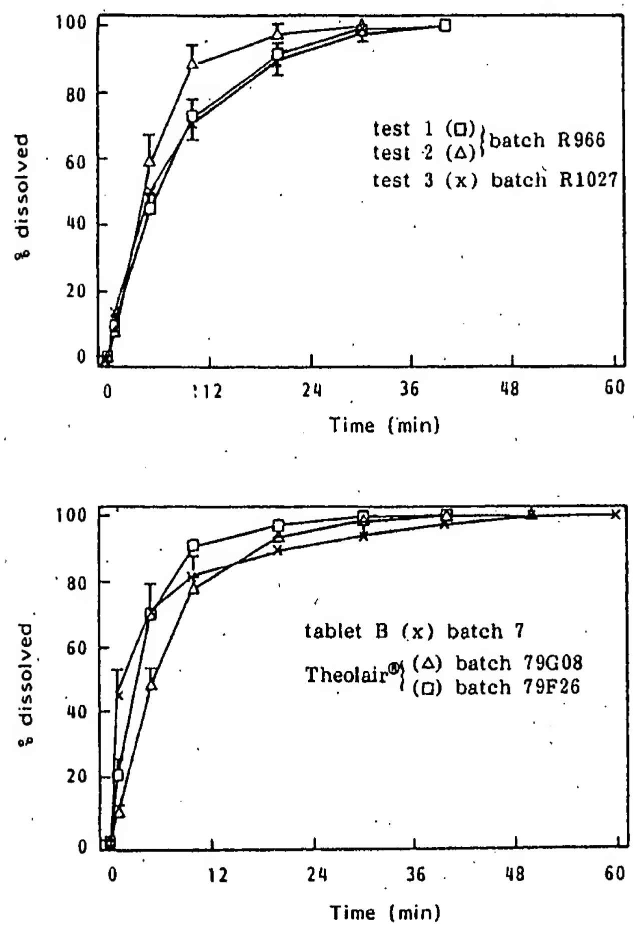

FIGURE 3

Dissolution profiles of theophylline tablets. Reproducibility of tablet A characteristics (top); comparison of Theolair® and tablet B (bottom).

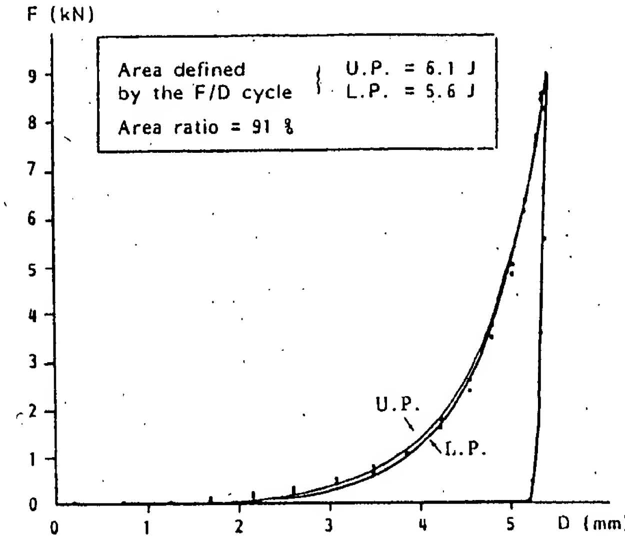

FIGURE 4

Force (F) applied on powder by the upper punch (U.P.) and stress transmitted through the powder to the lower punch (L.P.) as a function of the displacement of the upper punch (D).

TABLE 2

Effect of theophylline particle size on the properties of the tablets.

	Theophylline raw materials		
	commercial quality tablets A	selected quality tablets B	micronized quality tablets C
Compression force kN	8.8	6.8	6.7
Hardness daN ± S.D.	14.0 ± 0.6	11.9 ± 0.6	12.5 ± 0.7
Friability %	0.8	0.5	0.3
Mean weight ± S.D.	401.7 ± 3.4	403.3 ± 4.2	405.6 ± 3.1
Weight CV %	0.9	1.0	0.8
Disintegration time (sec)	30	28	40

TABLE 3

Pharmacokinetic parameters of theophylline from 3 dogs after single oral administration of an aqueous solution and 4 tablet formulations (250 mg theophylline) (mean ± S.D.)

Parameter	Aqueous solution	Tablet A (50 µm)	Tablet B (30 µm)	Tablet C (10 µm)	Theolair®
$t_{1/2}$abs (hr)	0.40 ± 0.47	0.73 ± 0.62	0.39 ± 0.25	0.52 ± 0.12	0.64 ± 0.30
t_{max} (hr)	1.7 ± 2.0	2.2 ± 1.6	1.8 ± 0.6	2.2 ± 0.7	2.8 ± 1.4
C_{max} (mg/l)	23.3 ± 9.6	18.4 ± 3.4	21.8 ± 1.2	16.6 ± 4.5	21.1 ± 0.7
$t_{1/2}$ β (hr)	6.8 ± 0.3	6.2 ± 2.2	6.2 ± 1.9	6.1 ± 1.4	5.7 ± 0.6
AUC $_\infty$ (mg.hr/l)	237 ± 75	163 ± 30	228 ± 66	181 ± 49	221 ± 67
F	--	0.71 ± 0.09	0.97 ± 0.16	0.77 ± 0.14	0.94 ± 0.19

tained are given in Table 3. The drug was rapidly absorbed after each administration, the half-life of absorption ($t_{1/2}$abs) was generally less than 1 hr and the time of the peak plasma concentration (t_{max}) occurred between 1 and 3 hr. Peak plasma concentrations ranged from 12.1 to 33.7 mg/l. The availability (F) of tablet B relative to the aqueous solution (0.97 ± 0.16) was superior to that of tablets A (0.71 ± 0.09) and C (0.77 ± 0.14) and was similar to that of Theolair® tablets (0.94 ± 0.19). The analysis of variance showed a significant difference between the AUC$_\infty$ values of the aqueous solution and tablet A ($p < 0.01$) or tablet C ($p < 0.05$). There was no significant difference between aqueous solution, tablet B and Theolair®.

No difference between formulations was found for the other pharmacokinetic parameters ($t_{1/2}$abs, t_{max}, C_{max}). Plasma elimination of theophylline showed half-lives ($t_{1/2}$β) of 6-7 hr for the five formulations tested. Westlake's test for the determination of the symmetrical confidence intervals (95% probability) where aqueous solution was chosen as the reference compound, showed a 21% confidence interval for tablet B, 23% for Theolair®, 47% and 39% for tablets A and C respectively. Confidence intervals near 20% or lower are generally accepted to establish the bioequivalence between two formulations. From these results it appears that aqueous solution, tablets B and Theolair® are bioequivalent, whilst the bioavailability of tablets A and C is lower.

Scale up

After completion of the "in vitro" and "in vivo" experiments, tablet B was chosen for the scale up study of the formulation. Firstly the influence of calcium phosphate, which is the major excipient (> 30%) of the formula was investigated. The characteristics of the formulation were not modified when four different batches of calcium phosphate were used as supplied from three different suppliers (table 4 and Fig. 5). The scale up study showed that for tablet B, granulation and compression characteristics were unaffected by the change in operating conditions during mixing which

TABLE 4

Effect of different batches of calcium phosphate (from different sources) on the properties of the tablets.

	Supplier A		Supplier B	Supplier C
	type A1	type A2		
Compression force kN	8.8	9.4	8.8	9.0
Hardness daN ± S.D.	15.5 ± 0.8	14.7 ± 0.6	15.8 ± 0.8	14.4 ± 0.5
Friability %	0.9	0.6	0.5	0.6
Mean weight ± S.D.	398.9 ± 3.6	402.0 ± 4.3	392.2 ± 4.6	400.9 ± 2.6
Weight CV %	0.9	1.1	1.2	0.7
Disintegration time (sec)	33	45	31	32

TABLE 5

Effect of the type and size of mixer on the properties of the tablets.

	Z arm mixer			Lödige mixer	
	1 l	5 l	30 l	50 l	130 l
Compression force kN	6.6	6.7	7.2	8.8	6.8
Hardness daN ± S.D.	12.4 ± 0.9	12.3 ± 0.7	13.2 ± 0.5	14.0 ± 0.6	13.7 ± 1.1
Friability %	0.5	0.5	0.5	0.8	0.9
Mean weight ± S.D.	405.2 ± 4.4	401.2 ± 2.2	402.0 ± 2.6	401.7 ± 3.4	404.5 ± 5.0
Weight CV %	1.1	0.5	0.6	0.9	1.2
Disintegration time (sec)	32	25	27	30	20

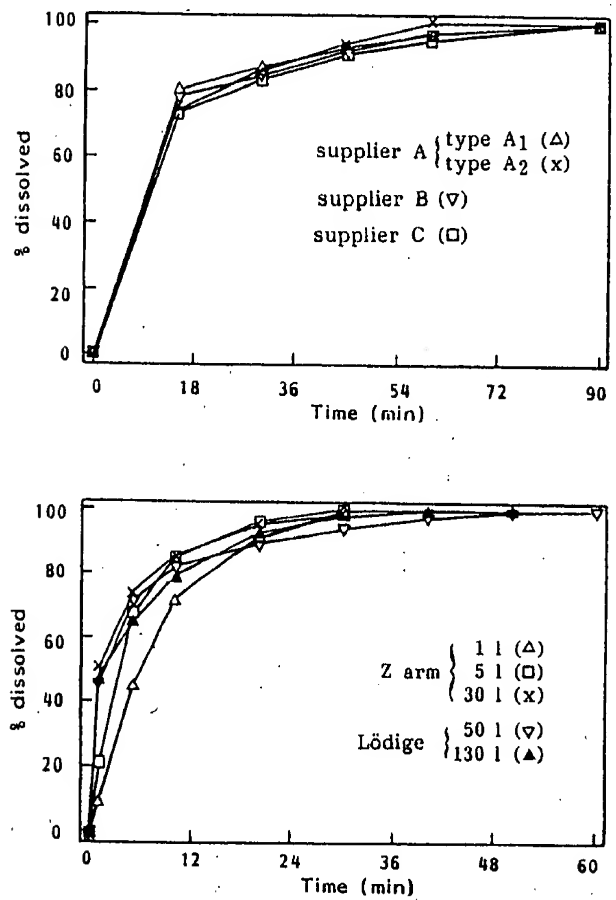

FIGURE 5

Effect of calcium phosphate (top) and mixer type (bottom) on dissolution profiles of tablets B.

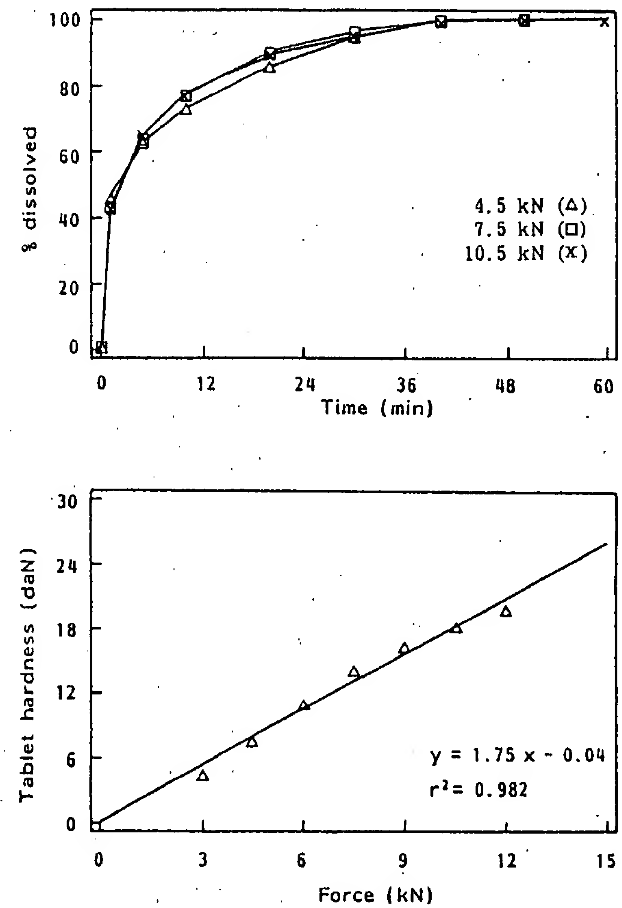

FIGURE 6

Effect of compression force on dissolution profiles (top) and tablet hardness (bottom).

were dictated by charges in size of the mixer used : Z arm mixers of 1, 5 and 30 liters, or Lödige mixers of 50 and 130 liters respectively. Table 5 and figure 5 show the results obtained for the characteristics of the granulated powders prepared, together with their dissolution kinetics. Powder tabletting was carried out under the same conditions using a rotating machine. Tablet hardness and dissolution kinetics were measured as a function of the compression force applied by the tabletting machine. The compression force was increased by steps of 1.5 kN, from 3 to 12 kN (a 7.5 kN value has previously been considered satisfactory for this formulation). In this range, tablet hardness increased linearly with the compression force, the relationship being expressed in the following equation obtained by linear regression :

$$y = 1.75\,x - 0.04 \quad (r^2 = 0.982,\ p < 0.01)$$

Dissolution kinetics remained unchanged for tablet compression force values between 4.5 and 10.5 kN (Fig. 6). It is important to underline that this formulation requires a relatively weak compression force for the preparation of the tablets (6.5 to 8.5 kN) which avoids a high consumption of energy when working under extreme conditions.

CONCLUSION

The first requirement for a new formulation is to make the drug completely available at the site of absorption. This will facilitate constant absorption of the drug and thereby provide a high systemic availability. When the absorption of the drug and not the release from the formulation represents the limiting step, the bioavailability of the formulation should be equivalent to that of an aqueous solution. In order to obtain a dissolution rate of theophylline higher than its rate of absorption, several parameters including particle size of raw materials, granulation, and tablet formula were investigated.

The studies on dissolution rate showed that the release of theophylline from tablet A (theophylline of commercial quality) and

tablet B (theophylline of selected particle size) was faster than from tablet C (micronized theophylline), whereas no difference was observed between tablets A and B. The "in vivo" study showed that only tablet B has the same bioavailability ($F = 0.97 \pm 0.16$) as an aqueous solution, whilst the bioavailability of tablet A ($F = 0.71 \pm 0.09$) and tablet C ($F = 0.77 \pm 0.14$) was lower than that of tablet B and the aqueous solution.

Therefore, "in vitro" experiments do not give an accurate prediction of the bioavailability of theophylline from a new formulation. Nevertheless, estimations from dissolution rate studies may enable the number of formulations tested to be reduced before proceeding to an "in vivo" evaluation.

The rapid and complete absorption of theophylline from tablet B and the bioequivalence with an aqueous solution have been confirmed in a study carried out in healthy adult subjects, after a single oral dose[8].

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DISSOLUTION OF SUPPOSITORIES III: EFFECT OF INSOLUBLE POLYVINYLPYRROLIDONE ON ACETAMINOPHEN RELEASE

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Abstract

Previously reported studies from this laboratory have demonstrated the usefulness of a new apparatus for suppository dissolution study. Acetaminophen suppositories gave slow release and it was posited that addition of a disintegrating agent commonly used in tablet manufacture would increase this release rate. To test the hypothesis, four PEG blends were used as bases as in the previous studies. Each contained 320 mg acetaminophen and 1%, 5%, or 10% of insoluble polyvinylpyrrolidone (Polyplasdone XL^{R}). One thousand milliliters of phosphate buffer, pH 8.0 to approximate rectal pH was employed as the dissolution media and maintained at 37.5°. A constant agitation rate of 25 and 50 rpm was used. Acetaminophen was assayed

* = Undergraduate Research Assistants

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YR 96
IN Seville, Esp
MF Univ.Seville
LG English
KW IN-VITRO/FT; IN-VIVO/FT; RAT/FT; SOLID/FT; MIXTURE/FT; BIOPHARM/FT; DISSOLUTION/FT; INTRAGASTRIC/FT; LAB-ANIMAL/FT.
1 OF 2.
01 TRIAMTERENE/OC; TRIAMTERENE/PH; TRIAMTERENE/DM; MIQUEL/FT; TRIAMTERE /RN; DIURETIC/FT; RELEASE/FT; BIOAVAILABILITY/FT; SOLUBILITY/FT; ABSORPTION /FT; PHARMACOKINETICS/FT; DIURETICS/FT; OC/FT; PH/FT; DM/FT
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2 OF 2.
02 POLYETHYLENE-GLYCOL/OC; ACO/FT; PEG/RN; AUXILIARY-INGREDIENT/FT; MOL /FT; WEIGHT/FT; PHARMACEUTICS/FT; OC/FT
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AU Asche H, Botta L, Rettig H, Weirich E G
TI **Influence of Formulation Factors on the Availability of Drugs from Topical Preparations**
SO Pharm-Acta-Helv (60, No. 8, 232-37, 1985)
YR 85

AN 89:5058 IPA Full-text
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AU Shah, J. C.; Chen, J. R.; Chow, D.
CS Dept. of Pharmaceutics, Coll. of Pharm., Univ. of Houston, 1441 Moursund St., Houston, TX 77030, USA
SO Pharmaceutical Research (USA), (May 1989) Vol. 6, pp. 408-412. 16 Refs. CODEN: PHREEB; ISSN: 0724-8741.
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SC 9 Pharmaceutics; 10 Drug Stability; 8 Biopharmaceutics
CC 10:00 Antineoplastic agents
IT Etoposide; solubility; and stability, relation, availability
IT Hydrogen ion concentration; etoposide; effects, solubility, stability
IT Partition coefficients; etoposide; relation, availability
IT Stability; etoposide; effects, pH, relation, availability
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IT Antineoplastic agents; etoposide; solubility, stability, relation, availability
IT Kinetics; dissolution; etoposide, in vitro, relation, availability
IT Drugs, availability; etoposide; relation, solubility, stability
IT Temperature; etoposide; stability, relation, availability
RN 33419-42-0 (Etoposide)
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TI **Dissolution properties and in vivo behaviour of triamterene in solid dispersions with polyethylene glycols**
SO Pharm-Acta-Helv (71, No. 4, 229-35, 1996)
YR 96
IN Seville, Esp
MF Univ.Seville
LG English
KW IN-VITRO/FT; IN-VIVO/FT; RAT/FT; SOLID/FT; MIXTURE/FT; BIOPHARM/FT; DISSOLUTION/FT; INTRAGASTRIC/FT; LAB-ANIMAL/FT.
1 OF 2.
01 TRIAMTERENE/OC; TRIAMTERENE/PH; TRIAMTERENE/DM; MIQUEL/FT; TRIAMTERE /RN; DIURETIC/FT; RELEASE/FT; BIOAVAILABILITY/FT; SOLUBILITY/FT; ABSORPTION /FT; PHARMACOKINETICS/FT; DIURETICS/FT; OC/FT; PH/FT; DM/FT
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1: Pharm Res 1989 May;6(5):408-12

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Shah JC, Chen JR, Chow D.

Department of Pharmaceutics, College of Pharmacy, University of Houston, Texas 77030.

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Pharmaceutical Research, Vol. 6, No. 5, 1989

Report

Preformulation Study of Etoposide: Identification of Physicochemical Characteristics Responsible for the Low and Erratic Oral Bioavailability of Etoposide

Jaymin C. Shah,[1,3] Jivn R. Chen,[2] and Diana Chow[1,4]

Received December 22, 1987; accepted December 29, 1988

Preformulation studies of etoposide, including pH–solubility profile, partition coefficient, pH–stability profile, and *in vitro* dissolution kinetics, were conducted to identify the responsible factor(s) for the low and erratic oral bioavailability of etoposide. A stability-indicating high-performance liquid chromatographic (HPLC) assay was used for drug monitoring. The equilibrium aqueous solubility of etoposide at 37°C was low, 148.5–167.25 μg/ml, and did not vary over the pH range of 2 to 6. The pH–stability profile indicated rapid degradation of etoposide at pH 1.3 and 10, with degradation half-lives of 2.88 and 3.83 hr, respectively, at 25°C. The half-life at pH 7.30 was 27.72 days. Maximum stability at 25°C was reached at pH 5 to 6.15, with half-lives of 63 and 49.5 days, respectively. The intrinsic dissolution rate, determined on a Wood's apparatus, was slow, 0.0094 mg/min/cm^2, while the etoposide partition coefficient between *n*-octanol and water was 9.94. Therefore, etoposide absorption appears to be dissolution rate limited rather than permeation rate limited. The low equilibrium aqueous solubility, slow intrinsic dissolution rate, and chemical instability at pH 1.3 could account for the low oral bioavailability.

KEY WORDS: etoposide; preformulation; pH–solubility; pH–stability; dissolution; partition coefficient.

INTRODUCTION

Etoposide, also known as VP-16-213, is a semisynthetic epipodophyllotoxin derivative (Fig. 1), active against a variety of malignancies (1). Etoposide is the most active single agent for the treatment of small-cell lung cancer and testicular carcinoma (2). The agent is given intravenously in a dose of 300–600 mg/m^2 (450–900 mg for an adult weighing 70 kg) over a period of 3–5 days. The treatment is repeated every alternate week until a beneficial effect is observed (3). The currently available dosage forms are nonaqueous i.v. parenteral solutions and oral soft gelatin capsules containing etoposide solution in a mixed solvent system. The i.v. administration of etoposide on a chronic basis is inconvenient for outpatients. In addition, etoposide precipitates from the parenteral solution as diluted with other i.v. fluids for infusion (4), and too rapid an infusion of etoposide precipitates hypotension of the patient (3). Therefore, an oral formulation is desired. However, the capsule formulation has a reported oral bioavailability of 50% (5). Several investigational oral formulations have been evaluated, namely, (a) hydrophilic, soft gelatin capsules containing etoposide solution (6), (b) lipophilic capsules of etoposide suspension (7), and (c) drinking ampoules (8). However, all these formulations yielded poor oral bioavailabilities (25–74%) with high intra- and interpatient variabilities in the rate and extent of etoposide absorption (9). Therefore, the development of a stable oral formulation with a higher and more reproducible oral bioavailability than the current one is desirable.

This study was intended to identify the possible physicochemical characteristics responsible for the low and erratic oral bioavailability of etoposide, for the purpose of establishing the basis for logical and effective approaches to modify the dosage form. The pH–solubility profile and pH–stability profile of etoposide were established, with the pH range encountered in the gastrointestinal tract (pH 1.3–8). The pH dependence of the solubility and chemical stability of the drug was determined. In addition, the *in vitro* dissolution kinetics of etoposide was evaluated using a Wood's apparatus (10). The possibility of dissolution rate-limiting absorption of etoposide was verified (11). The *n*-octanol/water partition coefficient of etoposide was also determined.

MATERIALS AND METHODS

Chemicals

Etoposide was used as received from Bristol Myers

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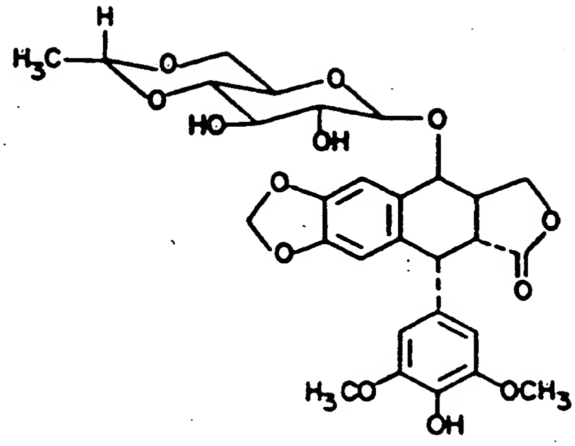
[4] To whom correspondence should be addressed at Department of Pharmaceutics, University of Houston, 1441 Moursund Street, Houston, Texas 77030.



Etoposide (VP-16-213)

Fig. 1. The chemical structure of etoposide.

(Syracuse, N.Y.). Hydrochloric acid, potassium chloride, sodium citrate, citric acid, acetic acid, sodium acetate, potassium monobasic phosphate, potassium dibasic phosphate, sodium hydroxide, and boric acid were all analytical grade. Acetonitrile of high-performance liquid chromatographic (HPLC) grade was used.

HPLC Assay

A stability-indicating HPLC assay was developed for etoposide (12) with a reversed-phase C8 column (5 μm, 15 cm × 4.6-mm i.d., Custom LC Inc., Houston, Tex.) and acetonitrile–acetic acid–water (27:1:72, pH 4.0) at a flow rate of 1.5 ml/min as the mobile phase. Etoposide was monitored at 230 nm and the detection limit was 0.05 μg/ml. Methoxypsoralen was used as the internal standard.

Preparation of Buffers

All buffers used, pH 1.3–10, as listed in Tables I and II, had concentrations of 0.1 *M*. The buffers used for the pH–stability study had ionic strengths adjusted to 0.5 with KCl.

pH–Solubility Profile

An excessive amount (about 20 mg) of etoposide was agitated with 10 ml of each buffer for 48 hr at 37°C in a water bath. One-milliliter samples were taken at 24 and 48 hr, respectively, filtered through 45-μm membrane filters (Gellman), and subjected to HPLC assay.

pH–Stability Profile

Etoposide solutions of 100 μg/ml prepared in the buffers were maintained at 25°C in a water bath. The samples were taken at various time intervals and analyzed by HPLC until the remaining etoposide level was negligible. The log concentration of etoposide versus time profile was plotted to determine the degradation rate constants at all pH values. The pH–stability profile was constructed by plotting rate constant versus pH.

Table I. Solubilities of Etoposide at 37°C in Buffers of Various pH Values

Buffer	pH	Solubility (μg/ml), Mean ± SD[a]
0.1 *M* HCl	1.30	Extensive degradation of etoposide was observed.
0.1 *M* HCl/KCl	2.00	151.31 ± 16.64*
0.1 *M* Na citrate/ citric acid	3.00	167.25 ± 16.67
Distilled water	4.50	147.50 ± 1.75
0.1 *M* Na acetate/ acetic acid	5.00	153.22 ± 10.18
0.1 *M* $KHPO_4/KH_2PO_4$	6.00	149.58 ± 9.73
0.1 *M* $KHPO_4/KH_2PO_4$	7.40	125.93 ± 19.41**
0.1 *M* $KHPO_4/KH_2PO_4$	8.00	116.44 ± 11.95** Etoposide degradation was observed after 48 hr
0.1 *M* Na borate/ boric acid	10.00	Extensive degradation of etoposide was observed

[a] N = 3, 24-hr data.
* Statistically no significant difference in solubilities from pH 2 to pH 6 at $P = 0.05$ by ANOVA.
** Statistically significant difference in solubilities from pH 6 to pH 8 at $P = 0.05$ by ANOVA.

Drug Dissolution Kinetics

About 25 mg of etoposide was compressed on a Carver press (Model C, Fred S. Carver Inc.) into a disk 6 mm in diameter and then mounted on a rotating shaft of a Wood's apparatus. The disk was rotated at 100 rpm in 30 ml of distilled water at room temperature. The distance of the disk from the bottom of the beaker was kept constant at 2 cm. Samples (100 μl) were taken at various time intervals up to

Table II. First-Order Degradation Half-Life ($t_{1/2}$) of Etoposide at 25°C in Buffers of Various pH Values

Buffer[a]	pH	$t_{1/2}$ (days), mean ± SD[b]
0.1 *M* HCl	1.30	0.12 ± 0.002
0.1 *M* HCl/KCl	2.03	1.19 ± 0.127
0.1 *M* Na citrate/ citric acid	3.05	8.15 ± 0.192
0.1 *M* Na acetate/ acetic acid	5.00	63.00 ± 5.730*
0.1 *M* $KHPO_4/KH_2PO_4$	6.15	49.50 ± 3.536
0.1 *M* $KHPO_4/KH_2PO_4$	7.30	27.72 ± 2.218
0.1 *M* $KHPO_4/KH_2PO_4$	8.00	5.97 ± 0.257
0.1 *M* Na borate/ boric acid	10.00	0.16 ± 0.011

[a] All buffers had concentrations of 0.1 *M*, and the ionic strengths had been adjusted to 0.5 with KCl.
[b] $N = 3$.
* Statistically no significant difference in half-lives at pH 5 and 6.15 by Student's *t* test at $P = 0.05$.

70 hr, filtered through 45-μm membrane filters, and analyzed by HPLC.

The data obtained were analyzed using the Noyes–Whitney equation (13) as shown below.

$$\frac{dc}{dt} = \frac{D * A * (C_s - C)}{h * V} \tag{1}$$

where dc/dt is the dissolution rate, D is the diffusion coefficient (cm^2/min), A is the surface area of the disk (cm^2), C_s is the aqueous solubility (mg/ml), C is the concentration of etoposide (mg/ml), h is the thickness of the diffusion layer (cm), and V is the volume of the dissolution medium (ml).

Under sink conditions (C is less than 20% of C_s), Eq. (1) is simplified as follows (13):

$$\frac{dc}{dt} = \frac{D * A * C_s}{h * V} \tag{2}$$

which, on rearrangement, leads to

$$\frac{dc * V}{dt * A} = \frac{D * C_s}{h} = \text{intrinsic dissolution rate} \tag{3}$$

Partition Coefficient

The *n*-octanol and water were presaturated with each other in amber-colored bottles for 24 hr. Five milliliters each of the two presaturated solvents was mixed together with 10 mg of etoposide in a screw-capped tube on a rotatory mixer at 25°C. Samples were taken at 6, 12, and 24 hr. The *n*-octanol and water layers of the samples were analyzed separately for etoposide by HPLC.

Statistical Analysis

The effects of pH on the solubility of etoposide were analyzed by one-way ANOVA at $P = 0.05$. To determine the pH of maximum stability, the degradation constants at pH 5 and 6.15 were compared by Student's t test at the $P = 0.05$ level.

RESULTS

pH–Solubility Profile

The solubilities of etoposide at 37°C in various buffers of pH's ranging from 1.30 to 10 are reported in Table I. Extensive degradation of etoposide was observed at pH 1.30 and 10, which precluded the measurement of equilibrium solubilities. The pH–solubility profile of etoposide is shown in Fig. 2. The difference in solubilities from pH 2 to pH 6 was insignificant but those at pH 7.4 and 8 were significantly lower than the rest as determined by ANOVA at $P = 0.05$. The apparent solubility decreased with increasing pH above pH 6, along with increasing etoposide degradation, as reflected by the increasing peak heights of the degradation products in the chromatograms (Fig. 3).

pH–Stability Profile

The log etoposide concentration versus time profiles

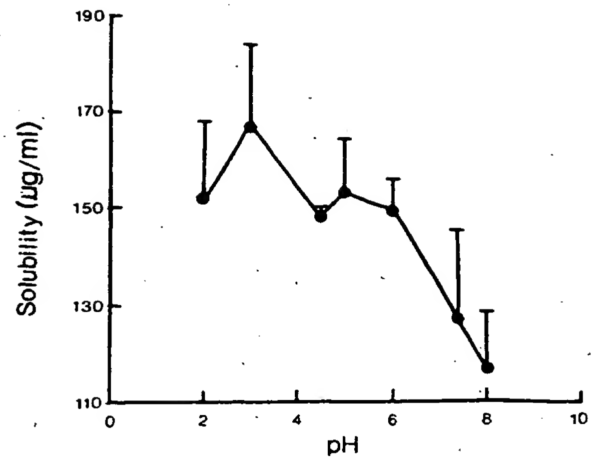


Fig. 2. The pH–solubility profile of etoposide at 37°C. Each point represents the mean of three observations with standard deviation bar.

were constructed. The linear curves of the plots at all pH's indicated first-order degradation. Degradation rate constants obtained from the slopes of the curves were used to determine the half-lives at various pH's (Table II). The pH–stability profile of etoposide is shown in Fig. 4. The degradations were extremely rapid under highly acidic and alkaline conditions. The degradation half-lives were 2.88 and 3.83 hr at pH 1.30 and pH 10, respectively, while pH 5–6.15 was the pH range of maximal stability, with degradation half-lives of 63 and 49.5 days, respectively. The slopes of the pH–rate profile on the acidic and basic sides were −0.70 and 0.68, respectively; therefore, the degradation of etoposide is not specific acid or base catalyzed (14).

Drug Dissolution Kinetics

Complete dissolution profiles in three separate runs

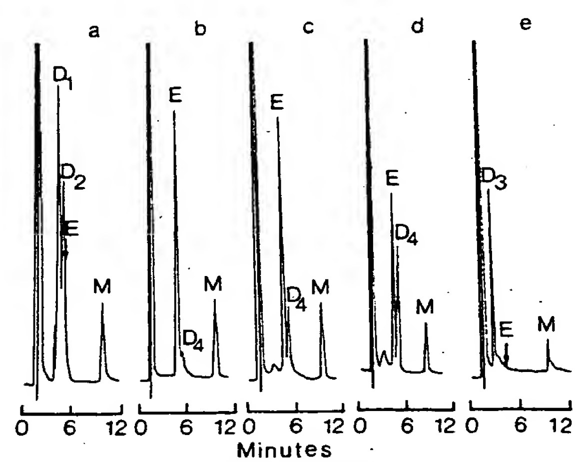


Fig. 3. Typical chromatograms of etoposide (E) solubility samples after 48 hr at (a) pH 1.3, (b) pH 6.0, (c) pH 7.4, (d) pH 8.0, and (e) pH 10.0. Methoxypsoralen (M) is the internal standard; D1, D2, D3, and D4 are different degradation products of etoposide.

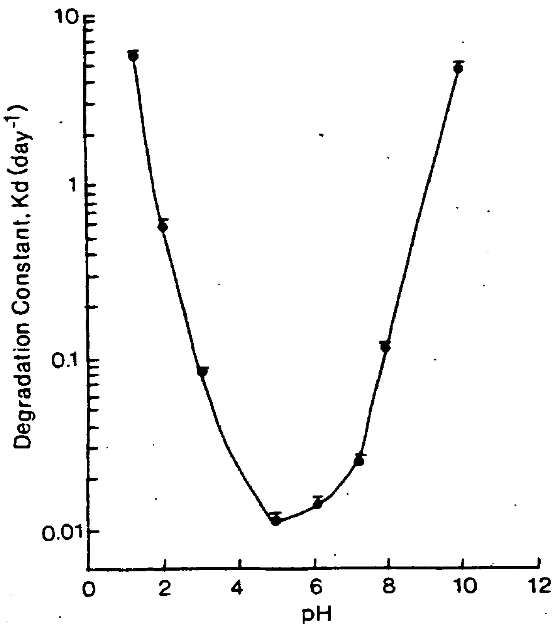


Fig. 4. The pH–stability profile of etoposide at room temperature. Each point represents the mean value of three observations with standard deviation bar.

were constructed until saturation of etoposide (0.1 mg/ml) was achieved as shown in Fig. 5. The dissolution kinetics of etoposide can be described by the Noyes–Whitney equation [Eq. (1)]. The curves were linear when etoposide concentrations were less than 20% of the equilibrium solubility of etoposide (Fig. 5, inset). The slope of the linear portion determined the dissolution rate as described in Eq. (2). The intrinsic dissolution rates were calculated according to Eq. (3) and are listed in Table III.

Partition Coefficient

The equilibrium partition of etoposide between *n*-octanol and water phases was achieved in 12 hr. The partition coefficient (o/w) was 9.94 ± 0.095 at 25°C (N = 3). No degradation products of etoposide were observed by HPLC during the partition coefficient study.

DISCUSSION

Etoposide solubilities ranged from 116.44 to 167.25 μg/ml over the pH range 1.3 to 8. Insufficient aqueous solubility of a drug has been known to yield poor or erratic absorption with large inter- and intrasubject variations in blood levels. Kaplan (11) found that potential bioavailability problems are often present when the aqueous solubility of a drug is less than 10 mg/ml (1%). The extremely low aqueous solubility of etoposide may be responsible for its poor and erratic oral absorption.

In addition, the orally administered drug needs to be stable during its transit through the gastrointestinal tract of various pH's ranging from 1 to 8. Etoposide is most stable in the pH range of 5–6.15 and rapidly degrades at pH <2.03 and pH >8. The half-life of etoposide at pH 1.30 was 2.85 hr. The rapid degradation of etoposide in gastric fluid could also account for its low oral bioavailability. An enteric coating of etoposide may prevent the acidic degradation and effectively improve the oral bioavailability.

Significant statistical correlations between drug absorption and dissolution rate have been reported for many drugs, such as digoxin, prednisone, and acetaminophen (15). Drugs having intrinsic dissolution rates less than 1.0 mg/min/cm^2 at 37°C frequently have bioavailability problems, because the absorption is limited by the dissolution rate (11). Digoxin,

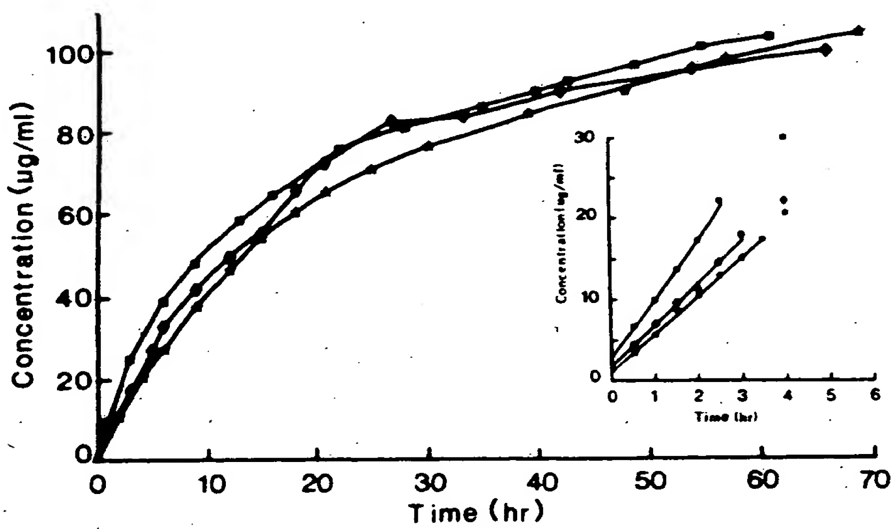


Fig. 5. Intrinsic dissolution profiles of etoposide in three separate dissolution experiments. The inset depicts the dissolution profile of etoposide under sink conditions (concentrations of etoposide less than 20% of aqueous solubility).

Table III. Dissolution Rates of Etoposide at 25°C

Dissolution experiment No.	Dissolution rate, dc/dt (μg/ml/hr)	Intrinsic dissolution rate, $(D/h) \cdot C_s$ (mg/min/cm²)
1	5.94	0.0105
2	5.27	0.0093
3	4.80	0.0085
Mean	5.34	0.0094
(SD)[a]	(0.57)	(0.0010)

[a] $N = 3$.

various erythromycin esters, and different hydrates of ampicillin are examples of drugs with dissolution rate-limiting absorption (16). The intrinsic dissolution rate of etoposide was 0.0094 mg/min/cm² at 25°C, and although it increases with temperature, its magnitude is far less than 1.0 mg/min/cm² at 37°C. Therefore, dissolution rate-limited absorption of etoposide may also contribute to the observed low oral bioavailability.

The correlation between the partition coefficient and the rate and extent of absorption of a drug has been reported (15). However, the absorption of etoposide may not be permeation rate limited, because the partition coefficient of etoposide was 9.94 at 25°C, reflecting its high lipophilicity.

In conclusion, the low aqueous solubility, slow intrinsic dissolution rate, and rapid degradation at pH 1.30 of etoposide may all account for the low and erratic bioavailability of the drug. Therefore, approaches to increase the aqueous solubility and dissolution rate of etoposide and to employ an enteric coating to prevent acidic degradation in gastric fluid may effectively improve the oral bioavailability of etoposide.

ACKNOWLEDGMENTS

This work was partially supported by a Sigma Xi Graduate Research Award (J.C.S.). It was presented in part at the 1st National Meeting of the American Association of Pharmaceutical Scientists, Washington, D.C., November 1986.

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FT; PHARM-PREP/FT.

'DM; SICORTEN/PH;
SONE/RC; 131/PI; CONC
:/FT; CREAM/FT;
HUMAN/FT;
FT; PERCUTANEOUS/FT;
ITRO/FT; URINE/FT;
IMAL/FT; CORTICOSTEROIDS

AUXILIARY-INGREDIENT

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icin Microspheres

E/FT; MICROSPHERE/FT;
ACEUTICS/FT.

01 INDOMETACIN/DM; 025/PI; 333/PI; CONC/FT; BLOOD-PLASMA/FT; ELIMINATION
/FT; URINE/FT; BIOAVAILABILITY/FT; PELLET/FT; CAPSULE/FT;
ANTIINFLAMMATORIES/FT; ANTIPYRETICS/FT; ANTIRHEUMATICS/FT; PROSTAGLANDIN-
ANTAGONISTS/FT; INDOMETAC/RN; DM/FT.
2 OF 2.
02 POLYACRYLATE/OC; EUDRAGIT-RL/OC; EUDRAGIT-RS/OC; MATRIX/FT; AUXILIARY-
INGREDIENT/FT; POLYACRYL/RN; OC/FT

AB In human subjects, bioavailability of controlled release indomethacin (IN) as microspheres (formulated with Eudragit RL and RS) was greater than that of pelletized IN following repeated p.o. use, but the difference was not significant. Methods Microspheres containing 56.3% IN were prepared using Eudragit RL and RS in equal quantities, by the o/w emulsion solvent diffusion technique. In 3 treatment periods at least 7 days apart, 12 male volunteers each received (a) conventional 25 mg IN capsules in 3 doses of 2 capsules at 6 hr intervals; (b) 75 mg IN pellets b.i.d. ; and (c) 75 mg micronized IN b.i.d. Plasma and urinary IN was determined by HPLC. Results Mean Cmax, Tmax, half-life in plasma, AUC (0-32 hr) AUC ratio (capsules = 100%), and urinary recovery (as % of dose) for IN microspheres were 3.2 ug /ml, 5.8 hr, 8.2 hr, 104.6 ug/ml.hr, 94.6%, and 22.3%, respectively, and for IN pellets were 3.1 ug/ml, 4.0 hr, 10.4 hr, 93.8 ug/ml.hr, 84.8%, and 20.2%, respectively. (WS).

AN 90-48371 900000
ISL Order Copy

[redacted] Tutsch Kendra D, Arzoomanian Rhoda Z, Alberti Dona, Binger Kim, Feierabend Chris, Dresen Amy, Marnocha Rebecca, Pluda James, Wilding George
IN University of Wisconsin Comprehensive Cancer Center, Madison, Wisconsin 53792, USA

TI **Phase I and pharmacokinetic study of a micronized formulation of carboxyamidotriazole, a calcium signal transduction inhibitor: toxicity, bioavailability and the effect of food**

SO Clinical cancer research, 2002 Jan, VOL: 8 (1), P: 86-94
ISN 1078-0432
YR 2002

DT Clinical-Trial, Clinical-Trial-Phase-I, Journal-Article
LG English
RN 0 (Antineoplastic-Agents);
0 (Calcium-Channel-Blockers);
0 (Capsules);
0 (Gels);
0 (Triazoles);
7440-70-2 (Calcium);
99519-84-3 (carboxyamido-triazole)

KW ADULT;
AGED;
AGED-80-AND-OVER;
ANTINEOPLASTIC-AGENTS/AE (adverse effects), *PK (pharmacokinetics);
BIOLOGICAL-AVAILABILITY;
CALCIUM/ME (metabolism);
CALCIUM-CHANNEL-BLOCKERS/AE (adverse effects), *PK (pharmacokinetics);
CAPSULES;
DIET-THERAPY;
DRUG-ADMINISTRATION-SCHEDULE;
FEMALE;
GELS;
HEMATOPOIESIS/DE (drug effects);
HUMAN;
INTESTINAL-ABSORPTION;
MALE;
MAXIMUM-TOLERATED-DOSE;

MIDDLE-AGE;
NEOPLASMS/BL (blood), *DT (drug therapy);
NERVOUS-SYSTEM/DE (drug effects);
SUPPORT-U-S-GOVT-P-H-S;
TRIAZOLES/AE (adverse effects), *PK (pharmacokinetics)

AB PURPOSE: This Phase I study was conducted to evaluate the toxicity profile and determine the maximum tolerated dose (MTD) of an oral micronized formulation of the signal transduction inhibitor carboxyamidotriazole (CAI) . Bioavailability of the micronized formulation relative to a gelatin capsule (gelcap) formulation was assessed. The effects of food intake and timing on CAI steady-state plasma concentrations (C(ss)) were also investigated. EXPERIMENTAL DESIGN: Patients received continuous daily CAI (28-day cycles). Starting dose was 150 mg/m(2) daily and escalations were by 50 mg/m(2) increments. The first three patients enrolled were given test doses of the original gelcap formulation and two different micronized formulations to determine relative bioavailability. Toxicity and pharmacokinetic assessments were performed weekly. Additional cohorts were added after MTD determination to assess the effect of food intake and duration of fast on CAI C(ss). RESULTS: **The micronized formulation was absorbed more slowly than the gelcap formulation.** Twenty-nine patients were enrolled in the dose-escalation portion of the study. After dose escalation to 300 mg/m(2), dose-limiting neurotoxicities occurred including reversible vision loss in two patients. Other toxicities were mild. The final MTD was 150 mg/m(2). Pharmacokinetics appeared linear with significant inter- and intrapatient variability. Patients with C(ss) of > or = 4.0 mg/liter were more likely to have neurotoxicity. Nine patients with renal cell cancer and one with hepatocellular cancer had prolonged stable disease. CAI plasma concentrations were higher when taken with food. CONCLUSIONS: Micronized CAI was well tolerated at the MTD of 150 mg/m(2). Higher doses were limited by significant neurotoxicity. The variability in CAI pharmacokinetics may be partially attributable to concomitant food intake and timing of the dose. Grant ID: M01-RR01386, Acronym: RR, Agency: NCRR Grant ID: P30CA14520, Acronym: CA, Agency: NCI Grant ID: U01-CA62491, Acronym: CA, Agency: NCI.

AN 11801543 Completed 20020416
ISL Order Copy

1993

[redacted] R E, Isley W L, Ogrinc F G, Klobucar T R
IN Upjohn Company, Kalamazoo, Michigan

TI **Efficacy and safety of reformulated, micronized glyburide tablets in patients with non-insulin-dependent diabetes mellitus: a multicenter, double-blind, randomized trial**

Clinical therapeutics, 1993 Sep-Oct, VOL: 15 (5), P: 788-96
0149-2918
1993

Clinical-Trial, Journal-Article, Multicenter-Study, Randomized-Controlled-Trial
English
0 (Blood-Glucose);
0 (Hemoglobin-A-Glycosylated);
0 (Tablets);
10238-21-8 (Glyburide)
ADULT;
AGED;
AGED-80-AND-OVER;
BLOOD-GLUCOSE/AN (analysis);
CHEMISTRY-PHARMACEUTICAL;
DIABETES-MELLITUS-NON-INSULIN-DEPENDENT/*DT (drug therapy);
DOUBLE-BLIND-METHOD;

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MIDDLE-AGE;
NEOPLASMS/BL (blood), *DT (drug therapy);
NERVOUS-SYSTEM/DE (drug effects);
SUPPORT-U-S-GOVT-P-H-S;
TRIAZOLES/AE (adverse effects), *PK (pharmacokinetics)

AB PURPOSE: This Phase I study was conducted to evaluate the toxicity profile and determine the maximum tolerated dose (MTD) of an oral micronized formulation of the signal transduction inhibitor carboxyamidotriazole (CAI) . Bioavailability of the micronized formulation relative to a gelatin capsule (gelcap) formulation was assessed. The effects of food intake and timing on CAI steady-state plasma concentrations (C(ss)) were also investigated. EXPERIMENTAL DESIGN: Patients received continuous daily CAI (28-day cycles). Starting dose was 150 mg/m(2) daily and escalations were by 50 mg/m(2) increments. The first three patients enrolled were given test doses of the original gelcap formulation and two different micronized formulations to determine relative bioavailability. Toxicity and pharmacokinetic assessments were performed weekly. Additional cohorts were added after MTD determination to assess the effect of food intake and duration of fast on CAI C(ss). RESULTS: **The micronized formulation was absorbed more slowly than the gelcap formulation.** Twenty-nine patients were enrolled in the dose-escalation portion of the study. After dose escalation to 300 mg/m(2), dose-limiting neurotoxicities occurred including reversible vision loss in two patients. Other toxicities were mild. The final MTD was 150 mg/m(2). Pharmacokinetics appeared linear with significant inter- and intrapatient variability. Patients with C(ss) of > or = 4.0 mg/liter were more likely to have neurotoxicity. Nine patients with renal cell cancer and one with hepatocellular cancer had prolonged stable disease. CAI plasma concentrations were higher when taken with food. CONCLUSIONS: Micronized CAI was well tolerated at the MTD of 150 mg/m(2). Higher doses were limited by significant neurotoxicity. The variability in CAI pharmacokinetics may be partially attributable to concomitant food intake and timing of the dose. Grant ID: MO1-RR01386, Acronym: RR, Agency: NCRR Grant ID: P30CA14520, Acronym: CA, Agency: NCI Grant ID: UO1-CA62491, Acronym: CA, Agency: NCI.
AN 11801543 Completed 20020416
ISL Order Copy

[redacted] R F, Isley W L, Ogrinc F G, Klobucar T R
IN Upjohn Company, Kalamazoo, Michigan
TI **Efficacy and safety of reformulated, micronized glyburide tablets in patients with non-insulin-dependent diabetes mellitus: a multicenter, double-blind, randomized trial**
SO Clinical therapeutics, 1993 Sep-Oct, VOL: 15 (5), P: 788-96
ISN 0149-2918
YR 1993
DT Clinical-Trial, Journal-Article, Multicenter-Study, Randomized-Controlled-Trial
LG English
RN 0 (Blood-Glucose);
0 (Hemoglobin-A-Glycosylated);
0 (Tablets);
10238-21-8 (Glyburide)
KW ADULT;
AGED;
AGED-80-AND-OVER;
BLOOD-GLUCOSE/AN (analysis);
CHEMISTRY-PHARMACEUTICAL;
DIABETES-MELLITUS-NON-INSULIN-DEPENDENT/*DT (drug therapy);
DOUBLE-BLIND-METHOD;

AU Arias M J, Gines J M, Moyano J R, Rabasco A M
TI **Dissolution properties and in vivo behaviour of triamterene in solid dispersions with polyethylene glycols**
SO Pharm-Acta-Helv (71, No. 4, 229-35, 1996)

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Agency: NCI.

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FEMALE;
GLYBURIDE/*AD (administration & dosage), AE (adverse effects);
HEMOGLOBIN-A-GLYCOSYLATED/AN (analysis);
HUMAN;
MALE;
MIDDLE-AGE;
TABLETS

AB The subjects were 206 patients (123 men, 83 women) with non-insulin-dependent diabetes mellitus, aged 33 to 80 years. For at least 4 weeks prior to the study each subject had been taking 5-mg tablets of original, nonmicronized glyburide (Micronase tablets) in doses of 5, 10, 15, or 20 mg daily. In a double-blind 12-week study, the subjects were randomly assigned to continue receiving 5-mg tablets of original glyburide or to substitute 3-mg tablets of reformulated, micronized glyburide (Glynase PresTab tablets) for the original tablets. Glyburide tablets had been reformulated to improve their bioavailability. Baseline mean fasting serum glucose levels in the groups taking reformulated and original glyburide were 169.3 and 168.3 mg/dl, respectively; at study end point, their respective serum glucose levels were 186.0 and 177.0 mg/dl. **The differences between groups were not significant;** in both groups, however, end point glucose levels were significantly higher than baseline levels. Baseline hemoglobin A1C levels in the groups taking reformulated and original glyburide were both 7.6%; at study end point, hemoglobin A1C levels had improved slightly in each group to 7.4% and 7.5%, respectively. The differences between and within groups at end point were not significant. No between-group differences at baseline or at end point were found in mean levels of postprandial serum glucose, fasting C-peptide, or postprandial C-peptide. Medical events experienced by the subjects in the two groups were similar in nature and number. Changes in other laboratory test results, vital signs, and weight were not clinically meaningful.(ABSTRACT TRUNCATED AT 250 WORDS).

AN 08269445 Completed 20020101
ISL Order Copy

AU Clarke J M, Ramsay L E, Shelton J R, Tidd M J, Murray S, Palmer R F
TI **Factors influencing comparative bioavailability of spironolactone tablets**
SO Journal of pharmaceutical sciences, 1977 Oct, VOL: 66 (10), P: 1429-32
ISN 0022-3549
YR 1977
DT Journal-Article
LG English
RN 0 (Tablets);
52-01-7 (Spironolactone)
KW ADULT;
BIOLOGICAL-AVAILABILITY;
HUMAN;
MALE;
MIDDLE-AGE;
SOLUBILITY;
SPIRONOLACTONE/AD (administration & dosage), *ME (metabolism);
TABLETS;
TIME-FACTORS

AB The bioavailability of spironolactone from 10 tablet formulations, selected to provide a wide range of specifications and in vitro dissolution rates, was assessed from the plasma and urinary levels of its major unconjugated metabolite, canrenone, in a study of balanced incomplete block design using 11 healthy

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nacokinetics, 1991,

(blood), *PK

AB For poorly water soluble drugs, the dissolution process in biological fluids the rate limiting step in absorption. However, the utilization of some galenic processes such as solid dispersions (SD) leads to an improvement in quality and intensity of the drug gastro-intestinal absorption. In a previous work, the in vitro studies of the dissolution curves of both the pure micronized progesterone (MP) and the progesterone-PEG 6000 SD revealed marked increases in the progesterone dissolution rates for all the SD investigated compared to the pure MP. The aim of this work was to investigate the in vitro results after oral administration of the two pharmaceutical forms to menopaused volunteer women.
AN 01820877 Completed 20020101
ISL Order Copy

[redacted], Clabaut M, Daoust M, Orecchioni A M
TI **About a Pharmacokinetic Study of Progesterone in Comelts**
SO Eur-J-Drug-Metab-Pharmacokinet (15, No. 2, Suppl., Abstr.226, 1990)
YR 90
IN St.Etienne Rouvray, France
LG English
KW 1 OF 1.
01 PROGESTERONE/OC; PROGESTERONE/DM; POLYETHYLENE-GLYCOL/RC; IN-VITRO/FT; DISSOLUTION/FT; RATE/FT; SOLID/FT; DISPERSION/FT; MICRONIZED/FT; BIOPHARM/FT; IN-VIVO/FT; HUMAN/FT; POSTCLIMACTERIC/FT; P-O/FT; CONC/FT; BIOAVAILABILITY/FT; PHARMACOKINETICS/FT; ABSORPTION/FT; PROGESTOGENS/FT; PROGESTER/RN; OC/FT; DM/FT
AB In vitro dissolution rate of progesterone was faster from polyethylene glycol 6000 solid dispersions (SD) than from pure micronized progesterone (MP). In healthy postmenopausal women given p.o. SD and MP, SD gave higher Cmax (17.35 vs. 7.55 ng/ml), earlier Tmax (45 vs. 120 min) and increased 8 hr AUC (35 vs. 20 ng/ml/min). Results show that SD enhances p.o. bioavailability of progesterone by increasing both its dissolution kinetics and its GI absorption. (congress abstract). (YC).
AN 90-36530 900000
ISL Order Copy

AU Ettinger B
TI **Prevention of Osteoporosis: Treatment of Estradiol Deficiency**
SO Obstet-Gynecol (72, No. 5, Suppl., 12S-17S, 1988)
YR 88
IN San Francisco, California, United States
LG English
KW OSTEOPOROSIS/TR; OSTEOPATHY/TR; CLIMACTERIC/FT; REVIEW/FT; CASES/FT; IN-VIVO/FT; PROPHYLAXIS/FT.
1 OF 2.
01 MAIN-TOPIC/FT; TR/FT.
2 OF 2.
02 ESTRADIOL/TR; PROGESTERONE/TR; MEDROXYPROGESTERONE-ACETATE/TR; NORGESTREL/TR; NORETHISTERONE-ACETATE/TR; NORHYDROXYPROGESTERONE-CAPROATE/TR; CALCIUM-SALT/TR; CALCIFEROL/TR; ESTROGEN/FT; PROGESTOGEN/FT; TR/FT
AB The use of estradiol (E2) therapy to prevent osteoporosis is reviewed. The mechanisms of the osteoporotic process are considered and factors which may retard or enhance the efficacy of therapy, such as smoking or Ca supplementation are considered. It is noted that while Ca enhances the response to E2, Ca alone does not prevent osteoporosis. Whatever the form of E2 used, it appears that the aim should be to achieve E2 levels akin to those seen in the follicular phase of the menstrual cycle. The use of concomitant progestogens is also considered. Reference is made to 17-beta-E2 as both cream and micronized formulation, to

conjugated estrogens, to progesterone, medroxyprogesterone acetate, norgestrel, norethindrone acetate, 19-nor-17-beta-hydroxyprogesterone caproate, and vitamin D. Estrogen deficiency leads primarily to a loss of trabecular bone. This may decline by 5-8%/yr whereas cortical bone may decline by only 1-3%. The rate of bone loss tails off steadily after a period of 10-15 yr, but E2 deficiency may nevertheless be responsible for some 15-20% of the trabecular bone loss suffered, and for 10-15% of the loss of cortical bone. Minimum doses of E2 needed to prevent this loss are 1.5 mg of E2 cream, 2.0 mg of the micronized form and 0.6 mg of conjugated estrogens. For the last 2 formulations, these figures fall to 1.0 and 0.3 mg when Ca supplements are also used. Smoking affects the bioavailability of E2 and reduces its effectiveness by about 50%. Studies have consistently demonstrated that E2 reduces the risk of fractures and there are good grounds for starting E2 therapy early after the onset of menopause, especially in high-risk patients. Therapy should be continued for as long as is feasible; usually this means for the remainder of life. Therapy is still useful when started later in life, but the benefits are not as great. In older patients, combination with Ca and vitamin D may be useful. Often, progestogens are administered concomitantly to reduce the risk of gynecological problems (e.g. endometrial cancer) associated with E2 monotherapy. To date, there seems to be no convincing evidence that progestogens have any beneficial effects on bone mass, whatever their other advantages. (E59/JW).

AN 88-50904 880000
ISL Order Copy

[redacted]T, Calvert R T, Riley Bentham P
TI **Bioavailability of griseofulvin from a novel capsule formulation**
SO The Journal of pharmacy and pharmacology, 1978 Aug, VOL: 30 (8), P: 479-82
ISSN 0022-3573
1978
Journal-Article
English
0 (Capsules);
126-07-8 (Griseofulvin)
BIOLOGICAL-AVAILABILITY;
CAPSULES;
DRUG-COMPOUNDING;
GRISEOFULVIN/AD (administration & dosage), *ME (metabolism);
HUMAN;
KINETICS;
MALE;
SOLUBILITY;
TIME-FACTORS

AB The in vivo availability of griseofulvin from a novel formulation has been compared with the micronized powder. The formulation technique involves the conversion of the hydrophobic surface of the drug to a hydrophilic one by treatment with a film forming polymer. This enhances the wettability of the power, and increases its dissolution rate. The results of the in vivo study show the formulation technique has increased the rate and extent of bioavailability of griseofulvin when compared with the non-treated powder.

AN 00028393 Completed 20020101
ISL Order Copy

AU [redacted] H, Grote U
[redacted]lized Preparations of Griseofulvins. 2nd Communication: In-Vivo Release. (Ger.)

Feld

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.te, 19-nor-17-beta-
n D. Estrogen deficiency
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SO Pharm-Ind (48, No. 7, 837-40, 1986)
YR 86
IN Berlin, Germany, West
LG German
KW HUMAN/FT; IN-VIVO/FT; P-O/FT; COMB/FT; LYOPHILIZATION/FT; BIOPHARM/FT; FORMULATION/FT; PHARMACEUTICS/FT.
1 OF 2.
01 GRISEOFULVIN/OC; GRISEOFULVIN/DM; EGA-CHEMIE/FT; 025/PI; ALONE/FT; METABOLITE/FT; URINARY/FT; BIOAVAILABILITY/FT; IN-VITRO/FT; SOLUBILIZATION /FT; ELIMINATION/FT; FUNGICIDES/FT; ANTIBIOTICS/FT; GRISEOFUL/RN; OC/FT; DM /FT.
2 OF 2.
02 MANNITOL/OC; AUXILIARY-INGREDIENT/FT; PHARMACEUTICS/FT; DIURETICS/FT; LAXATIVES/FT; MANNITOL/RN; OC/FT

AB Bioavailability of p.o. freeze-dried griseofulvin (GF, EGA-Chemie) with mannitol in 6 volunteers was greater than that of a simple mixture of GF and mannitol, freeze-dried GF alone or micronized GF. Bioavailability of formulations correlated with in vitro solubilization. Formulation affects bioavailability of GF markedly. Methods 6 Men (24-34 yr old) received preparations containing 250 mg, GF in the form of micronized GF, GF with 9 fold excess of mannitol, lyophilized GF or GF colyophilized with a 9 fold excess of mannitol after a 12 hr fast. Urine was assayed for 6-desmethyl-GF spectrophotometrically. Results Mean bioavailability, relative to micronized GF (100%), was 136.5% for lyophilized GF, 153.7% for GF/mannitol mixture and 196.9% for lyophilized GF/mannitol. Relationships between in vitro solubilization of formulations after 30 min and in vivo excretion after 3 or 6 hr were almost linear. (U34 /KEW) (Lyophilisierte Zubereitungen des Griseofulvins. 2. Mitt.: In-Vivo-Freisetzung.).

AN 87-05897 870000
ISL Order Copy

AU Hargrove J T, Maxson W S, Wentz A C
IN Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, TN
TI **Absorption of oral progesterone is influenced by vehicle and particle size**
SO American journal of obstetrics and gynecology, 1989 Oct, VOL: 161 (4), P: 948-51
ISN 0002-9378
YR 1989
DT Journal-Article
LG English
RN 0 (Vehicles);
57-83-0 (Progesterone)
KW ABSORPTION;
ADMINISTRATION-ORAL;
BIOLOGICAL-AVAILABILITY;
FEMALE;
HUMAN;
MALE;
MIDDLE-AGE;
PARTICLE-SIZE;
PROGESTERONE/AD (administration & dosage), *BL (blood);
RADIOIMMUNOASSAY;
VEHICLES

AB The oral route of progesterone administration has long been considered impractical because of poor absorption and short biologic half-life. Recent reports suggest that micronization of progesterone enhances absorption and increases serum and tissue levels of progesterone. This study checks serum progesterone levels before and 0.5, 1, 2, 3, 4, and 6 hours after oral

administration of 200 mg of progesterone in seven subjects. Progesterone was plain milled, micronized, plain milled in oil, micronized in oil, or micronized in enteric-coated capsules. All patients exhibited a significant increase in serum progesterone levels after oral progesterone administration. Mean peak progesterone levels (30.3 +/- 7.0 ng/ml) (p less than 0.005) were achieved with micronized progesterone in oil at 2.0 +/-0.3 (p less than 0.05) hours after administration. Four types of oral progesterone had equivalent mean peak elevations and mean times to peak: plain milled, 9.6 +/- 2.5 ng/ml at 4.0 +/- 0.5 hours; micronized 13.2 +/-2.4 ng/ml at 3.2 +/- 0.4 hours; plain milled in oil, 11.3 +/- 3.0 ng/ml at 4.0 +/- 0.5 hours; and micronized in enteric-coated capsules, 11.2 +/- 3.0 ng/ml at 4.1 +/- 0.7 hours. Contrary to traditional teaching, these data show that significant serum progesterone levels can be achieved by oral administration. Absorption can be significantly improved by the physical characteristics of the progesterone and the vehicle used with oral administration.

AN 02801843 Completed 20020101

ISL Order Copy

AU [redacted] D, Guzelhan C, Crijns H`J M J, Peeters P A M, Persson P, Jonkman J H G

IN La Roche Ltd, Dept of Clinical Research (PRCP-D), Grenzacherstrasse, CH-4002 Basle, Switzerland

TI **Comparison of galenic formulations of orlistat (tetrahydrolipstatin). A pharmacological approach**

SO Drug Investigation, 1993, Vol/Iss/Pg. 5/1 (44-50)

ISN 0114-2402

1993

ENGLISH

Hartmann 1993

hoffmann la roche, Switzerland

tetrahydrolipstatin 96829-58-2; triacylglycerol-lipase 9001-62-1

Medical:
drug-formulation*;
abdominal-discomfort/side effect;
adult;
article;
comparative-study;
controlled-study;
fat-intake;
feces-incontinence/side effect;
human;
human-experiment;
lipid-absorption;
male;
oral-drug-administration.

Drug:
tetrahydrolipstatin*/adverse drug reaction, drug dose, pharmaceutics;
enzyme-inhibitor/drug dose, pharmaceutics;
feces-lipid;
lipase-inhibitor+/drug dose, pharmaceutics;
radioisotope;
triacylglycerol-lipase;
unclassified-drug

AB Orlistat (tetrahydrolipstatin) reduces absorption of dietary fat by inhibiting lipases in the gastrointestinal tract. Since conventional bioavailability testing by pharmacokinetic methods is meaningless, 2 capsule formulations containing orlistat as **micronised powder (A) or granules (B))** were compared using the following pharmacological end-points: faecal fat excretion after multiple 3-times-daily doses, and 14C-recovery in breath (breath test) and in faeces after single doses administered with

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pase 9001-62-1

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14C-triolein. The study was conducted in 12 hospitalised healthy male subjects at dose levels of 50 and 150 mg according to a balanced 4-way crossover scheme. The diet was standardised with an intake of 76 g fat per day. Orlistat was generally well tolerated. The few adverse events of moderate intensity were limited to the gastrointestinal tract and were consequences of the pharmacological action of the drug. At the 50 and 150 mg doses, respectively, mean faecal fat excretion (% of dietary fat intake) was 29.6 and 35.4% for capsule A, and 30.4 and 37.4% for capsule B. Mean 14C-recovery in faeces (% of 14C-dose) was 52.5 and 56.2% for A, and 50.5 and 62.9% for B. Mean cumulative 14C excretion in breath after 24 hours (% of 14C-dose) was 17.6 and 13.6% for A and 16.7 and 11.2% for B. At the 50 mg dose both capsules were pharmacologically equivalent. **At the 150 mg dose B showed a trend towards superior efficacy compared with A** (p = 0.09). The 150 mg doses were significantly more effective (p < 0.05) than the 50 mg doses. There were no significant carry-over effects. All investigated end-points yielded consistent results. The 14C-breath test proved to be a reliable and convenient method to assess fat absorption in relative terms and thus to compare galenic formulations of orlistat.

AN 1993045436 19930101
ISL Order Copy

AU Heyer K, Froemming K H
TI **Solidified Melts of Griseofulvin in Pluronic F68. II. In vivo release and Bioavailability.(Ger.).**
SO Dtsch-Apoth-Ztg (123, No. 18, 859-61, 1983)
YR 83
IN Berlin, Germany, West
LG German
KW SOLID/FT; SOLUTION/FT; FORMULATION/FT; PHARM-PREP/FT; PHARMACEUTICS/FT.
1 OF 2.
01 GRISEOFULVIN/OC; GRISEOFULVIN/DM; EGA-CHEMIE/FT; HUMAN/FT; RELEASE/FT; RATE/FT; BIOAVAILABILITY/FT; CONC/FT; URINE/FT; FUNGICIDES/FT; ANTIBIOTICS/FT; BIOPHARM/FT; GRISEOFUL/RN; OC/FT; DM/FT.
2 OF 2.
02 PLURONIC-F68/OC; WYANDOTTE/FT; SURFACTANTS/FT; POLYMER/FT; POLYALCOHOL/FT; OC/FT; 014/G8; 11H/G8; 110/G8; 115/G8; 116/G8; 12A/G8; 124/G8; 126/G8; 130/G8; 181/G8; 268/G8; PLURONF68/RN
AB A solidified melt of griseofulvin (EGA Chemie) in Pluronic F68 (Wyandotte) given to healthy volunteers resulted in increased total urinary excretion of 6-demethylgriseofulvin (DG) compared to the physical mixture and micronized griseofulvin. Faster griseofulvin release was also occurring. Methods 5 Healthy males (29-35 yr; 58-75 kg) received either micronized griseofulvin, 250 mg, its physical mixture with Pluronic F68 (20% antibiotic; 80% Pluronic F68) or a solid melt of griseofulvin in Pluronic F68. Cumulative urinary PG and its conjugate were followed over 72 hr. Results The maximum excretion rate of DG after the melt preparation (mean level 15.88 mg/hr) was significantly greater than after the micronized material (5.46 mg/hr) or the physical mixture (8.39 mg/hr). The rate of release of the drug from the physical mixture was not significantly greater than from the micronized material. Total cumulative excretion of DG after the melt preparation (67.22 mg) and the physical mixture (55.03 mg) was greater than after the micronized material (41.72 mg). Bioavailability of griseofulvin was improved by 26.1-29% by physical mixture and by 49.8 to 63.6% by melt preparations with Pluronic F68. (Schmelzeinbettungen des Griseofulvins in Pluronic F68. II. In-vivo -Wirkstofffreisetzung und Bioverfuegbarkeit.).
AN 83-29238 830000
ISL Order Copy

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P, Tillement J P
ic Acid in Humans
.30-34, 1983)

HUMAN/FT; P-O/FT; HALF-LIFE
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IM/FT; ANTIINFLAMMATORIES/FT;
PHARMACOKINETICS/FT; PHARM-

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IIZED/FT; DISSOLUTION/FT;
IRINE/FT; ELIMINATION/FT;
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carried out in 3 male Rhesus monkeys. A suspension of nonmicronized N in 5% tween 80/5% ethanol administered by gavage was used as a standard. Dosing procedures were standard except that the animals were sedated with 20 mg ketamine HCl given i.m. 5 min before dosage. Tablets were given 4 wk apart. Serum and urine levels of N were measured. The human bioavailability study on N was an open, 2-way, randomized, crossover, single-dose study in 20 healthy male volunteers. Results Weight and thickness variations and disintegration times were comparable between tablets made from micronized and nonmicronized N but the breaking strength of the former was greater. Uniformly higher concentrations in serum and urine showed more efficient absorption of micronized N in Rhesus monkeys. The levels were similar to those when N was administered as gavage. (W137/KD).

AN 87-24405 870000
ISL Order Copy

AU Kiortsis D N
IN D.N. Kiortsis, Laboratory of Physiology, University of Ioannina, Ioannina, Greece
TI **Micronized fenofibrate**
SO American Journal of Cardiovascular Drugs, 2002, Vol /Iss/Pg. 2/2 (134)
ISN 1175-3277
YR 2002
LG ENGLISH
RN fenofibrate 49562-28-9
KW Medical:
ischemic-heart-disease*/drug therapy, prevention;
dyslipidemia*/drug therapy;
drug-formulation;
cardiovascular-risk;
cholesterol-blood-level;
drug-targeting;
drug-bioavailability;
diabetes-mellitus;
triacylglycerol-blood-level;
human;
controlled-study;
note;
priority-journal.
Drug:
fenofibrate*/drug comparison, drug therapy, pharmaceutics, pharmacokinetics, pharmacology;
high-density-lipoprotein-cholesterol/endogenous compound;
hydroxymethylglutaryl-coenzyme-A-reductase-inhibitor/drug comparison, drug therapy, pharmacology;
triacylglycerol/endogenous compound
AN 2002197858 20020627
ISL Order Copy

[redacted], Takeuchi Y, Etoh A, Noda K
TI **Pharmacokinetics and bioavailability of diltiazem (CRD-401) in dog**
SO Arzneimittel-Forschung, 1977 Jul, VOL: 27 (7), P: 1424-8
ISN 0004-4172
YR 1977
DT Journal-Article
LG English
RN 0 (Benzazepines);
0 (Delayed-Action-Preparations);
0 (Solutions);
0 (Tablets);
42399-41-7 (Diltiazem)

Kohno

administration of 200 mg of progesterone in seven subjects. Progesterone was plain milled, micronized, plain milled in oil, micronized in oil, or micronized in enteric-coated capsules. All patients exhibited a significant increase in serum progesterone levels after oral progesterone administration. Mean peak progesterone levels (30.3 +/- 7.0 ng/ml) (p less than 0.005) were achieved with micronized progesterone in oil at 2.0 +/-0.3 (p less than 0.05) hours after administration. Four types of oral progesterone had equivalent mean peak elevations and mean times to peak: plain milled, 9.6 +/- 2.5 ng/ml at 4.0 +/- 0.5 hours; micronized 13.2 +/-2.4 ng/ml at 3.2 +/- 0.4 hours; plain milled in oil, 11.3 +/- 3.0 ng/ml at 4.0 +/- 0.5 hours; and micronized in enteric-coated capsules, 11.2 +/- 3.0 ng/ml at 4.1 +/- 0.7 hours. Contrary to traditional teaching, these data show that significant serum progesterone levels can be achieved by oral administration. Absorption can be significantly improved by the physical characteristics of the progesterone and the vehicle used with oral administration.

AN 02801843 Completed 20020101
ISL Order Copy

AU Hartmann D, Guzelhan C, Crijns H J M J, Peeters P A M, Persson P, Jonkman J H G
IN La Roche Ltd, Dept of Clinical Research (PRCP-D), Grenzacherstrasse, CH-4002 Basle, Switzerland
TI **Comparison of galenic formulations of orlistat (tetrahydrolipstatin). A pharmacological approach**
SO Drug Investigation, 1993, Vol/Iss/Pg. 5/1 (44-50)
ISN 0114-2402
YR 1993
LG ENGLISH
MF hoffmann la roche, Switzerland
RN tetrahydrolipstatin 96829-58-2; triacylglycerol-lipase 9001-62-1
KW Medical:
drug-formulation*;
abdominal-discomfort/side effect;
adult;
article;
comparative-study;
controlled-study;
fat-intake;
feces-incontinence/side effect;
human;
human-experiment;
lipid-absorption;
male;
oral-drug-administration.
Drug:
tetrahydrolipstatin*/adverse drug reaction, drug dose, pharmaceutics;
enzyme-inhibitor/drug dose, pharmaceutics;
feces-lipid;
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radioisotope;
triacylglycerol-lipase;
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AB Orlistat (tetrahydrolipstatin) reduces absorption of dietary fat by inhibiting lipases in the gastrointestinal tract. Since conventional bioavailability testing by pharmacokinetic methods is meaningless, 2 capsule formulations containing orlistat as **micronised powder (A) or granules (B)**) were compared using the following pharmacological end-points: faecal fat excretion after multiple 3-times-daily doses, and 14C-recovery in breath (breath test) and in faeces after single doses administered with

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